DESCRIPTION

METHOD FOR DIAGNOSING TESTICULAR SEMINOMAS

FIELD OF THE INVENTION

The invention relates to methods of diagnosing testicular seminomas.

PRIORITY INFOMATION

This application claims priority to United States Provisional Application Serial No.60/414,677, filed Seprember 30, 2002.

BACKGROUND OF THE INVENTION

Although testicular germ cell tumors (TGCTs) account for around 1-2% of all cancers in males, they are the most common cancers found in males aged 20 to 40 year-old age group(1), and the incidence has been markedly increasing over the past several decades(2,3). TGCTs are divided into two main histological types, the seminoma, which resembles the undifferentiated germ cells and the nonseminoma, which can resemble both embryonic and extra-embryonic tissues due to their ability to differentiate down either pathway(7). Seminoma is the most common histologic testis tumor in TGCTs and account for approximately 60% to 65% of all TGCTs(8). Currently, Alpha-fetoprotein (AFP), human beta-subunit chorionic gonadotropin (HCGβ) and lactic dehydrogenase (LDH) have been used as diagnostic tumor markers of TGCTs (9). However, a specific tumor marker of seminoma without syncytiotrophoblastic giant cells has not been identified.

cDNA microarray technologies have enabled to obtain comprehensive profiles of gene expression in normal and malignant cells, and compare the gene expression in malignant and corresponding normal cells (Okabe et al., Cancer Res 61:2129-37 (2001); Kitahara et al., Cancer Res 61: 3544-9 (2001); Lin et al., Oncogene 21:4120-8 (2002); Hasegawa et al., Cancer Res 62:7012-7 (2002)). This approach enables to disclose the complex nature of cancer cells, and helps to understand the mechanism of carcinogenesis. Identification of genes that are deregulated in tumors can lead to more precise and accurate diagnosis of individual cancers, and to develop novel therapeutic targets (Bienz and Clevers, Cell 103:311-20 (2000)). To disclose mechanisms underlying tumors from a genome-wide point of view, and discover target molecules for diagnosis and development of novel therapeutic drugs, the present inventors have

been analyzing the expression profiles of tumor cells using a cDNA microarray of 23040 genes (Okabe et al., Cancer Res 61:2129-37 (2001); Kitahara et al., Cancer Res 61:3544-9 (2001); Lin et al., Oncogene 21:4120-8 (2002); Hasegawa et al., Cancer Res 62:7012-7 (2002)).

Studies designed to reveal mechanisms of carcinogenesis have already facilitated identification of molecular targets for anti-tumor agents. For example, inhibitors of farnexyltransferase (FTIs) which were originally developed to inhibit the growth-signaling pathway related to Ras, whose activation depends on posttranslational farnesylation, has been effective in treating Ras-dependent tumors in animal models (He et al., Cell 99:335-45 (1999)). Clinical trials on human using a combination or anti-cancer drugs and anti-HER2 monoclonal antibody, trastuzumab, have been conducted to antagonize the proto-oncogene receptor HER2/neu; and have been achieving improved clinical response and overall survival of breast-cancer patients (Lin et al., Cancer Res 61:6345-9 (2001)). A tyrosine kinase inhibitor, STI-571, which selectively inactivates bcr-abl fusion proteins, has been developed to treat chronic myelogenous leukemias wherein constitutive activation of bcr-abl tyrosine kinase plays a crucial role in the transformation of leukocytes. Agents of these kinds are designed to suppress oncogenic activity of specific gene products (Fujita et al., Cancer Res 61:7722-6 (2001)). Therefore, gene products commonly up-regulated in cancerous cells may serve as potential targets for developing novel anti-cancer agents.

It has been demonstrated that CD8+ cytotoxic T lymphocytes (CTLs) recognize epitope peptides derived from tumor-associated antigens (TAAs) presented on MHC Class I molecule, and lyse tumor cells. Since the discovery of MAGE family as the first example of TAAs, many other TAAs have been discovered using immunological approaches (Boon, Int J Cancer 54: 177-80 (1993); Boon and van der Bruggen, J Exp Med 183: 725-9 (1996); van der Bruggen et al., Science 254: 1643-7 (1991); Brichard et al., J Exp Med 178: 489-95 (1993); Kawakami et al., J Exp Med 180: 347-52 (1994)). Some of the discovered TAAs are now in the stage of clinical development as targets of immunotherapy. TAAs discovered so far include MAGE (van der Bruggen et al., Science 254: 1643-7 (1991)), gp100 (Kawakami et al., J Exp Med 180: 347-52 (1994)), SART (Shichijo et al., J Exp Med 187: 277-88 (1998)), and NY-ESO-1 (Chen et al., Proc Natl Acad Sci USA 94: 1914-8 (1997)). On the other hand, gene products which had been demonstrated to be specifically overexpressed in tumor cells, have been shown to be recognized as targets inducing cellular immune responses. Such gene products include p53 (Umano et al., Brit J Cancer 84: 1052-7 (2001)), HER2/neu (Tanaka et al., Brit J Cancer 84: 94-9 (2001)), CEA (Nukaya et al., Int J Cancer 80: 92-7 (1999)), and so on.

In spite of significant progress in basic and clinical research concerning TAAs (Rosenbeg et al., Nature Med 4: 321-7 (1998); Mukherji et al., Proc Natl Acad Sci USA 92: 8078-82 (1995); Hu et al., Cancer Res 56: 2479-83 (1996)), only limited number of candidate TAAs for the treatment of adenocarcinomas, including colorectal cancer, are available. TAAs abundantly expressed in cancer cells, and at the same time which expression is restricted to cancer cells would be promising candidates as immunotherapeutic targets. Further, identification of new TAAs inducing potent and specific antitumor immune responses is expected to encourage clinical use of peptide vaccination strategy in various types of cancer (Boon and can der Bruggen, J Exp Med 183: 725-9 (1996); van der Bruggen et al., Science 254: 1643-7 (1991); Brichard et al., J Exp Med 178: 489-95 (1993); Kawakami et al.,J Exp Med 180: 347-52 (1994); Shichijo et al., J Exp Med 187: 277-88 (1998); Chen et al., Proc Natl Acad Sci USA 94: 1914-8 (1997); Harris, J Natl Cancer Inst 88: 1442-5 (1996); Butterfield et al., Cancer Res 59: 3134-42 (1999); Vissers et al., Cancer Res 59: 5554-9 (1999); van der Burg et al., J Immunol 156: 3308-14 (1996); Tanaka et al., Cáncer Res 57: 4465-8 (1997); Fujie et al., Int J Cancer 80: 169-72 (1999); Kikuchi et al., Int J Cancer 81: 459-66 (1999); Oiso et al., Int J Cancer 81: 387-94 (1999)).

It has been repeatedly reported that peptide-stimulated peripheral blood mononuclear cells (PBMCs) from certain healthy donors produce significant levels of IFN-γ in response to the peptide, but rarely exert cytotoxicity against tumor cells in an HLA-A24 or –A0201 restricted manner in ^{51}Cr-release assays (Kawano et al., Cance Res 60: 3550-8 (2000); Nishizaka et al., Cancer Res 60: 4830-7 (2000); Tamura et al., Jpn J Cancer Res 92: 762-7 (2001)). However, both of HLA-A24 and HLA-A0201 are one of the popular HLA alleles in Japanese, as well as Caucasian (Date et al., Tissue Antigens 47: 93-101 (1996); Kondo et al., J Immunol 155: 4307-12 (1995); Kubo et al., J Immunol 152: 3913-24 (1994); Imanishi et al., Proceeding of the eleventh International Hictocompatibility Workshop and Conference Oxford University Press, Oxford, 1065 (1992); Williams et al., Tissue Antigen 49: 129 (1997)). Thus, antigenic peptides of carcinomas presented by these HLAs may be especially useful for the treatment of carcinomas among Japanese and Caucasian. Further, it is known that the induction of low-affinity CTL in vitro usually results from the use of peptide at a high concentration, generating a high level of specific peptide/MHC complexes on antigen presenting cells (APCs), which will effectively activate these CTL (Alexander-Miller et al., Proc Natl Acad Sci USA 93: 4102-7 (1996)).

PYRIN-containing Apaf-1-like proteins (PYPAFs) are recently identified proteins (37). It has been reported that 14 PYPAFs genes exist in *Homo sapiens* (38). All of PYPAF proteins which contains leucine-rich repeat, PYRIN, NACHT and NACHT-associated domains were

thought to function in apoptotic and inflammatory signaling pathways. PYRIN domain at the N terminus has been reported to be associated with protein-protein interaction (38). In addition, NACHT domain has sequence homology with the nucleotide-binding motif of apoptotic protease-activating factor-1 (APAF-1), and are predicted to bind ATP(37). However, PYRIN-containing Apaf-1-like proteins have never been involved in tumorigenesis.

SUMMARY OF THE INVENTION

The invention is based on the discovery of a pattern of gene expression correlated with testicular seminomas (TS). The genes that are differentially expressed in TS are collectively referred to herein as "TS nucleic acids" or "TS polynucleotides" and the corresponding encoded polypeptides are referred to as "TS polypeptides" or "TS proteins."

Accordingly, the invention features a method of diagnosing or determining a predisposition to TS in a subject by determining an expression level of a TS-associated gene in a patient derived biological sample, such as tissue sample. By TS associated gene is meant a gene that is characterized by an expression level which differs in a cell obtained from a testicular germ cell tumor cell compared to a normal cell. A normal cell is one obtained from testis tissue. A TS-associated gene is one or more of TS 1-939. An alteration, *e.g.*, increase or decrease of the level of expression of the gene compared to a normal control level of the gene indicates that the subject suffers from or is at risk of developing TS.

By normal control level is meant a level of gene expression detected in a normal, healthy individual or in a population of individuals known not to be suffering from TS. A control level is a single expression pattern derived from a single reference population or from a plurality of expression patterns. For example, the control level can be a database of expression patterns from previously tested cells. A normal individual is one with no clinical symptoms of TS and no family history of TS.

An increase in the level of TS 1-346 detected in a test sample compared to a normal control level indicates the subject (from which the sample was obtained) suffers from or is at risk of developing TS. In contrast, a decrease in the level of TS 347-939 detected in a test sample compared to a normal control level indicates said subject suffers from or is at risk of developing TS.

Alternatively, expression of a panel of TS-associated genes in the sample is compared to a TS control level of the same panel of genes. By TS control level is meant the expression profile of the TS-associated genes found in a population suffering from TS.

Gene expression is increased or decreased 10%, 25%, 50% compared to the control level. Alternately, gene expression is increased or decreased 0.1, 0.2, 1, 2, 5, 10 or more fold compared to the control level. Expression is determined by detecting hybridization, *e.g.*, on an array, of a TS-associated gene probe to a gene transcript of the patient-derived tissue sample.

The patient derived tissue sample is any tissue from a test subject, e.g., a patient known to or suspected of having TS. For example, the tissue contains a testicular germ cell tumor cell. For example, the tissue is a cell from testis.

The invention also provides a TS reference expression profile of a gene expression level of two or more of TS 1-346. Alternatively, the invention provides a TS reference expression profile of the levels of expression of two or more of TS 1-346 or TS 347-939.

The invention further provides methods of identifing an agent that inhibits or enhances the expression or activity of a TS-associated gene, e.g TS 1-939 by contacting a test cell expressing a TS associated gene with a test agent and determining the expression level of the TS associated gene. The test cell is a testis cell such as a testis cell from a testicular germ cell tumor. A decrease of the level compared to a normal control level of the gene indicates that the test agent is an inhibitor of the TS-associated gene and reduces a symptom of TS. Alternatively, an increase of the level or activity compared to a normal control level or activity of the gene indicates that said test agent is an enhancer of expression or function of the TS associated gene and reduces a symptom of TS, e.g, TS 347-939.

The invention also provides a kit with a detection reagent which binds to two or more TS nucleic acid sequences or which binds to a gene product encoded by the nucleic acid sequences. Also provided is an array of nucleic acids that binds to two or more TS nucleic acids.

Therapeutic methods include a method of treating or preventing TS in a subject by administering to the subject an antisense composition. The antisense composition reduces the expression of a specific target gene, e.g., the antisense composition contains a nucleotide, which is complementary to a sequence selected from the group consisting of TS 1-346. Another method includes the steps of administering to a subject an short interfering RNA (siRNA) composition. The siRNA composition reduces the expression of a nucleic acid selected from the group consisting of TS 1-346. We demonstrated that *PYPAF3* was commonly up-regulated

in testicular seminomas and knock down of *PYPAF3* transcript by small interference RNA (siRNA) inhibited cell growth of testicular germ cell tumor cells.

In yet another method, treatment or prevention of TS in a subject is carried out by administering to a subject a ribozyme composition. The nucleic acid-specific ribozyme composition reduces the expression of a nucleic acid selected from the group consisting of TS 1-346. Other therapeutic methods include those in which a subject is administered a compound that increases the expression of TS 347-939 or activity of a polypeptide encoded by TS 347-939. Furthermore, TS can be treated by administering a protein encoded by TS 347-939. The protein may be directly administered to the patient or, alternatively, may be expressed *in vivo* subsequent to being introduced into the patient, for example, by administering an expression vector or host cell carrying the down-regulated marker gene of interest. Suitable mechanisms for in vivo expression of a gene of interest are known in the art.

The invention also includes vaccines and vaccination methods. For example, a method of treating or preventing TS in a subject is carried out by administering to the subject a vaccine containing a polypeptide encoded by a nucleic acid selected from the group consisting of TS 1-346 or an immunologically active fragment such a polypeptide. An immunologically active fragment is a polypeptide that is shorter in length than the full-length naturally-occurring protein and which induces an immune response. For example, an immunologically active fragment at least 8 residues in length and stimulates an immune cell such as a T cell or a B cell. Immune cell stimulation is measured by detecting cell proliferation, elaboration of cytokines (e.g., IL-2), or production of an antibody.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

One advantage of the methods described herein is that the disease is identified prior to detection of overt clinical symptoms. Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts photograph of a DNA agarose gel showing expression of representative 28 genes and TUBA examined by semi-quantitative RT-PCR using cDNA prepared from amplified RNA. The first 11 lanes show the expression level of the genes in a different TS patient. The last lane shows the expression level of each gene in testis from a normal individual. Gene symbols are noted for the genes.

Figure 2A depicts expression of *PYPAF3* examined by semi-quantitative RT-PCR in 8 testicular seminoma clinical samples (No. 1, 2, 7, 8, 9, 10, 11 and 13), normal human testis (TES), heart (HER), lung (LUN), liver (LIV), kidney (KID), brain (BRA) and bone marrow (BM). Expression of *TUBA3* served as an internal control. Figure 2B depicts northern analysis with a multiple-tissue blot using *PYPAF3* cDNA fragment as a probe.

Figure 3 depicts sub-cellular localization of myc-tagged PYPAF3 protein. Myc-tagged PYPAF3 protein extracts of COS-7 cells transfected with pcDNA3.1-myc/His-PYPAF3 plasmids. The transfected cells were stained with mouse anti-myc monoclonal antibody and visualized by FITC-conjugated anti-mouse IgG secondary antibody. Nuclei were counter-stained with DAPI.

Figure 4 depicts growth-inhibitory effects of small-interference RNAs (siRNAs) designed to reduce expression of PYPAF3 in testicular germ cell tumor line Tera-2. (A) Semi-quantitative RT-PCR showing suppression of endogenous expression of PYPAF3 in testicular germ cell tumor line Tera-2 at two weeks (cultures in selective medium containing neomycin after introduction of siRNAs into testicular germ cell tumor line Tera-2 cells. β2-microgloblin (*β2MG*) was used as an internal control. (B) Colony-formation assay demonstrating a decrease in the numbers of colonies by knockdown of PYPAF3 (Si1, Si2, Si3, Si4, and Si5) in testicular germ cell tumor line Tera-2 cells at two weeks, compared to psiU6BX-*EGFP (siEGFP)*, psiU6BX-Luciferase (siLuc) as controls. (C) MTT assay of testicular germ cell tumor line Tera-2 cells treated with either psiU6BX-PYPAF3 (Si1, Si2, Si3, Si4, and Si5), psiU6BX-*EGFP (siEGFP)*, psiU6BX-Luciferase (siLuc) by using Cell Counting Kit-8 at one week. These experiments were carried out three times as well.

DETAILED DESCRIPTION

The present invention is based in part on the discovery of changes in expression patterns

of multiple nucleic acid sequences in cells from testis of patients with TS. The differences in gene expression were identified by using a comprehensive cDNA microarray system.

Using a cDNA microarray containing 23,040 genes, comprehensive gene-expression profiles of 13 patients were constructed. Certain genes are expressed at low or high levels in TS patients. In the process candidate molecular markers were selected with the potential of detecting cancer-related proteins in serum or sputum of patients, and some potential targets for development of signal-suppressing strategies in human testicular cancer were discovered.

The differentially expressed genes identified herein are used for diagnostic purposes as markers of TS and as gene targets, the expression of which is altered to treat or alleviate a symptom of TS.

The genes whose expression levels are modulated (*i.e.*, increased or decreased) in TS patients are summarized in Tables 3,4 and are collectively referred to herein as " TS-associated genes " TS-associated genes "TS nucleic acids" or "TS polynucleotides" and the corresponding encoded polypeptides are referred to as "TS polypeptides" or "TS proteins." Unless indicated otherwise, "TS" is meant to refer to any of the sequences disclosed herein. (*e.g.*, TS 1-939). The genes have been previously described and are presented along with a database accession number.

By measuring expression of the various genes in a sample of cells, TS is diagnosed. Similarly, by measuring the expression of these genes in response to various agents, and agents for treating TS can be identified.

The invention involves determining (*e.g.*, measuring) the expression of at least one, and up to all the TS sequences listed in Tables 3,4. Using sequence information provided by the GeneBank™ database entries for the known sequences the TS associated genes are detected and measured using techniques well known to one of ordinary skill in the art. For example, sequences within the sequence database entries corresponding to TS sequences, are used to construct probes for detecting TS RNA sequences in, *e.g.*, northern blot hybridization analyses. Probes include at least 10, 20, 50, 100, 200 nucleotides of a reference sequence. As another example, the sequences can be used to construct primers for specifically amplifying the TS sequences in, *e.g*, amplification-based detection methods such as reverse-transcription based polymerase chain reaction.

Expression level of one or more of the TS sequences in the test cell population, *e.g.*, a patient derived tissues sample is then compared to expression levels of the some sequences in a

reference population. The reference cell population includes one or more cells for which the compared parameter is known, *i.e.*, TS cells or non-TS cells.

Whether or not a pattern of gene expression in the test cell population compared to the reference cell population indicates TS or a predisposition thereto depends upon the composition of the reference cell population. For example, if the reference cell population is composed of non-TS cells, a similar gene expression pattern in the test cell population and reference cell population indicates the test cell population is non-TS. Conversely, if the reference cell population is made up of TS cells, a similar gene expression profile between the test cell population and the reference cell population indicates that the test cell population includes TS cells.

A level of expression of a TS marker gene in a test cell population is considered altered in levels of expression if its expression level varies from the reference cell population by more than 1.0, 1.5, 2.0, 5.0, 10.0 or more fold from the expression level of the corresponding TS sequence in the reference cell population.

Differential gene expression between a test cell population and a reference cell population is normalized to a control nucleic acid, *e.g.* a housekeeping gene. For example, a control nucleic acid is one which is known not to differ depending on the endometriotic or non-endometriotic state of the cell. Expression levels of the control nucleic acid in the test and reference nucleic acid can be used to normalize signal levels in the compared populations. Control genes include β-actin, glyceraldehyde 3- phosphate dehydrogenase or ribosomal protein P1.

The test cell population is compared to multiple reference cell populations. Each of the multiple reference populations may differ in the known parameter. Thus, a test cell population may be compared to a second reference cell population known to contain, *e.g.*, TS cells, as well as a second reference population known to contain, *e.g.*, non-TS cells (normal cells). The test cell is included in a tissue type or cell sample from a subject known to contain, or to be suspected of containing, TS cells.

The test cell is obtained from a bodily tissue or a bodily fluid, *e.g.*, biological fluid (such as blood or urine). For example, the test cell is purified from a tissue. Preferably, the test cell population comprises an epithelial cell. The epithelial cell is from tissue known to be or suspected to be a TS.

Cells in the reference cell population are derived from a tissue type as similar to test cell. Optionally, the reference cell population is a cell line, *e.g.* a TS cell line (positive control) or a

normal non-TS cell line (negative control). Alternatively, the control cell population is derived from a database of molecular information derived from cells for which the assayed parameter or condition is known.

The subject is preferably a mammal. The mammal can be, *e.g.*, a human, non-human primate, mouse, rat, dog, cat, horse, or cow.

Expression of the genes disclosed herein is determined at the protein or nucleic acid level using methods known in the art. For example, Northern hybridization analysis using probes which specifically recognize one or more of these sequences can be used to determine gene expression. Alternatively, expression is measured using reverse-transcription-based PCR assays, *e.g.*, using primers specific for the differentially expressed sequences. Expression is also determined at the protein level, *i.e.*, by measuring the levels of polypeptides encoded by the gene products described herein, or biological activity thereof. Such methods are well known in the art and include, e.g., immunoassays based on antibodies to proteins encoded by the genes. The biological activity of the proteins encoded by the genes are also well known.

Diagnosing TS

TS is diagnosed by measuring the level of expression of one or more TS nucleic acid sequences from a test population of cells, (*i.e.*, a patient derived biological sample). Preferably, the test cell population contains an epithelial cell, e.g., a cell obtained from testis tissue. Gene expression is also measured from blood or other bodily fluids such as urine. Other biological samples can be used for measuring the protein level. For example, the protein level in the blood, or serum derived from subject to be diagnosed can be measured by immunoassay or biological assay.

Expression of one or more of TS-associated genes, *e.g.*, TS 1-939 is determined in the test cell or biological sample and compared to the expression of the normal control level. A normal control level is an expression profile of TS-associated genes typically found in a population known not to be suffering from TS. An increase or a decrease of the level of expression in the patient derived tissue sample of the TS associated genes indicates that the subject is suffering from or is at risk of developing TS. For example, an increase in expression of TS 1-346 in the test population compared to the normal control level indicates that the subject is suffering from or is at risk of developing TS. Conversely, a decrease in expression of TS 347-939 in the test population compared to the normal control level indicates that the subject is suffering from or is at risk of developing TS.

When one or more of the TS -associated genes are altered in the test population compared

to the normal control level indicates that the subject suffers from or is at risk of developing TS. For example, at least 1%, 5%, 25%, 50%, 60%, 80%, 90% or more of the panel of TS-associated genes (TS 1-346, TS 347-939, or TS 1-939) are altered.

Identifying Agents that inhibit or enhance TS-associated gene expression

An agent that inhibits the expression or activity of a TS-associated gene is identified by contacting a test cell population expressing a TS associated up-regulated gene with a test agent and determining the expression level of the TS associated gene. A decrease in expression in the presence of the agent compared to the normal control level (or compared to the level in the absence of the test agent) indicates the agent is an inhibitor of a TS associated up-regulated gene and useful to inhibit TS.

Alternatively, an agent that enhances the expression or activity of a TS down-regulated associated gene is identified by contacting a test cell population expressing a TS associated gene with a test agent and determining the expression level or activity of the TS associated down-regulated gene. An increase of expression or activity compared to a normal control expression level or activity of the TS-associated gene indicates that the test agent augments expression or activity of the down-regulated TS associated gene.

The test cell population is any cell expressing the TS-associated genes. For example, the test cell population contains an epithelial cell, such as a cell is or derived from testis. For example, the test cell is an immortalized cell line derived from testicular germ cell tumor. Alternatively, the test cell is a cell, which has been transfected with a TS-associated gene or which has been transfected with a regulatory sequence (*e.g.* promoter sequence) from a TS-associated gene operably linked to a reporter gene.

Assessing efficacy of treatment of TS in a subject

The differentially expressed TS sequences identified herein also allow for the course of treatment of TS to be monitored. In this method, a test cell population is provided from a subject undergoing treatment for TS. If desired, test cell populations are obtained from the subject at various time points before, during, or after treatment. Expression of one or more of the TS sequences, in the cell population is then determined and compared to a reference cell population which includes cells whose TS state is known. The reference cells have not been exposed to the treatment.

If the reference cell population contains no TS cells, a similarity in expression between TS sequences in the test cell population and the reference cell population indicates that the treatment is efficacious. However, a difference in expression between TS sequences in the test population and a normal control reference cell population indicates the less favorable clinical outcome or prognosis.

By "efficacious" is meant that the treatment leads to a reduction in expression of a pathologically up-regulated gene, increase in expression of a pathologically down-regulated gene or a decrease in size, prevalence, or metastatic potential of testicular tumors in a subject. When treatment is applied prophylactically, "efficacious" means that the treatment retards or prevents TS from forming or retards, prevents, or alleviates a symptom of clinical TS. Assesment of testicular tumors are made using standard clinical protocols.

Efficaciousness is determined in association with any known method for diagnosing or treating TS. TS is diagnosed for example, by identifying symptomatic anomalies, *e.g.,* painless enlargement of the testis.

Selecting a therapeutic agent for treating TS that is appropriate for a particular individual

Differences in the genetic makeup of individuals can result in differences in their relative abilities to metabolize various drugs. An agent that is metabolized in a subject to act as an anti-TS agent can manifest itself by inducing a change in gene expression pattern in the subject's cells from that characteristic of an TS state to a gene expression pattern characteristic of a non- TS state. Accordingly, the differentially expressed TS sequences disclosed herein allow for a putative therapeutic or prophylactic inhibitor of TS to be tested in a test cell population from a selected subject in order to determine if the agent is a suitable inhibitor of TS in the subject.

To identify an inhibitor or enhancer of TS, that is appropriate for a specific subject, a test cell population from the subject is exposed to a therapeutic agent, and the expression of one or more of TS 1-939 sequences is determined.

The test cell population contains a TS cell expressing a TS associated gene. Preferably, the test cell is an epithelial cell. For example a test cell population is incubated in the presence of a candidate agent and the pattern of gene expression of the test sample is measured and compared to one or more reference profiles, *e.g.,* a TS reference expression profile or a non-TS reference expression profile.

A decrease in expression of one or more of the sequences TS 1-346 or an increase in expression of one or more of the sequences TS 347-939 in a test cell population relative to a reference cell population containing TS is indicative that the agent is therapeutic.

The test agent can be any compound or composition. For example, the test agents are immunomodulatory agents.

Screening assays for identifying therapeutic agents

The differentially expressed sequences disclosed herein can also be used to identify candidate therapeutic agents for treating a TS. The method is based on screening a candidate therapeutic agent to determine if it converts an expression profile of TS 1-939 sequences characteristic of a TS state to a pattern indicative of a non-TS state.

In the method, a cell is exposed to a test agent or a combination of test agents (sequentially or consequentially) and the expression of one or more TS 1-939 sequences in the cell is measured. The expression profile of the TS sequences in the test population is compared to expression level of the TS sequences in a reference cell population that is not exposed to the test agent.

An agent effective in stimulating expression of under-expressed genes, or in suppressing expression of over-expressed genes is deemed to lead to a clinical benefit such compounds are further tested for the ability to prevent endometrial cyst growth, *e.g.*, endometrial glands and/or stroma, in animals or test subjects.

In a further embodiment, the present invention provides methods for screening candidate agents which are potential targets in the treatment of TS. As discussed in detail above, by controlling the expression levels or activities of marker genes, one can control the onset and progression of TS. Thus, candidate agents, which are potential targets in the treatment of TS, can be identified through screenings that use the expression levels and activities of marker genes as indices. In the context of the present invention, such screening may comprise, for example, the following steps:

a) contacting a test compound with a polypeptide encoded by TS 1-939;

b) detecting the binding activity between the polypeptide and the test compound; and

c) selecting a compound that binds to the polypeptide

Alternatively, the screening method of the present invention may comprise the following steps:

a) contacting a candidate compound with a cell expressing one or more marker genes, wherein the one or more marker genes is selected from the group consisting of TS 1-

939; and

b) selecting a compound that reduces the expression level of one or more marker genes selected from the group consisting of TS 1-346, or elevates the expression level of one or more marker genes selected from the group consisting of TS 347-939.

Cells expressing a marker gene include, for example, cell lines established from TS; such cells can be used for the above screening of the present invention.

Alternatively, the screening method of the present invention may comprise the following steps:

a) contacting a test compound with a polypeptide encoded by selected from the group consisting of TS 1-939;

b) detecting the biological activity of the polypeptide of step (a); and

c) selecting a compound that suppresses the biological activity of the polypeptide encoded by TS 1-346 in comparison with the biological activity detected in the absence of the test compound, or enhances the the biological activity of the polypeptide encoded by TS 347-939 in comparison with the biological activity detected in the absence of the test compound.

A protein required for the screening can be obtained as a recombinant protein using the nucleotide sequence of the marker gene. Based on the information of the marker gene, one skilled in the art can select any biological activity of the protein as an index for screening and a measurement method based on the selected biological activity.

Alternatively, the screening method of the present invention may comprise the following steps:

a) contacting a candidate compound with a cell into which a vector comprising the transcriptional regulatory region of one or more marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the one or more marker genes are selected from the group consisting of TS 1-939

b) measuring the activity of said reporter gene; and

c) selecting a compound that reduces the expression level of said reporter gene when said marker gene is an up-regulated marker gene selected from the group consisting of TS 1-346 or that enhances the expression level of said reporter gene when said marker gene is a down-regulated marker gene selected from the group consisting of TS 347-939, as compared to a control.

Suitable reporter genes and host cells are well known in the art. The reporter construct required for the screening can be prepared by using the transcriptional regulatory region of a marker gene. When the transcriptional regulatory region of a marker gene has been known to those skilled in the art, a reporter construct can be prepared by using the previous sequence information. When the transcriptional regulatory region of a marker gene remains unidentified, a nucleotide segment containing the transcriptional regulatory region can be isolated from a genome library based on the nucleotide sequence information of the marker gene.

The compound isolated by the screening is a candidate for drugs that inhibit the activity of the protein encoded by marker genes and can be applied to the treatment or prevention of TS.

Moreover, compound in which a part of the structure of the compound inhibiting the activity of proteins encoded by marker genes is converted by addition, deletion and/or replacement are also included in the compounds obtainable by the screening method of the present invention.

When administrating the compound isolated by the method of the invention as a pharmaceutical for humans and other mammals, such as mice, rats, guinea-pigs, rabbits, cats, dogs, sheep, pigs, cattle, monkeys, baboons, and chimpanzees, the isolated compound can be directly administered or can be formulated into a dosage form using known pharmaceutical preparation methods. For example, according to the need, the drugs can be taken orally, as sugar-coated tablets, capsules, elixirs and microcapsules, or non-orally, in the form of injections of sterile solutions or suspensions with water or any other pharmaceutically acceptable liquid. For example, the compounds can be mixed with pharmaceutically acceptable carriers or media, specifically, sterilized water, physiological saline, plant-oils, emulsifiers, suspending agents, surfactants, stabilizers, flavoring agents, excipients, vehicles, preservatives, binders, and such, in a unit dose form required for generally accepted drug implementation. The amount of active ingredients in these preparations makes a suitable dosage within the indicated range acquirable.

Examples of additives that can be mixed to tablets and capsules are, binders such as gelatin, corn starch, tragacanth gum and arabic gum; excipients such as crystalline cellulose; swelling agents such as corn starch, gelatin and alginic acid; lubricants such as magnesium stearate; sweeteners such as sucrose, lactose or saccharin; and flavoring agents such as peppermint, Gaultheria adenothrix oil and cherry. When the unit-dose form is a capsule, a liquid carrier, such as an oil, can also be further included in the above ingredients. Sterile composites for injections can be formulated following normal drug implementations using vehicles such as distilled water used for injections.

Physiological saline, glucose, and other isotonic liquids including adjuvants, such as D-sorbitol, D-mannnose, D-mannitol, and sodium chloride, can be used as aqueous solutions for injections. These can be used in conjunction with suitable solubilizers, such as alcohol, specifically ethanol, polyalcohols such as propylene glycol and polyethylene glycol, non-ionic surfactants, such as Polysorbate 80 (TM) and HCO-50.

Sesame oil or Soy-bean oil can be used as a oleaginous liquid and may be used in conjunction with benzyl benzoate or benzyl alcohol as a solubilizer and may be formulated with a buffer, such as phosphate buffer and sodium acetate buffer; a pain-killer, such as procaine hydrochloride; a stabilizer, such as benzyl alcohol and phenol; and an anti-oxidant. The prepared injection may be filled into a suitable ampule.

Methods well known to one skilled in the art may be used to administer the pharmaceutical composition of the present inevntion to patients, for example as intraarterial, intravenous, or percutaneous injections and also as intranasal, transbronchial, intramuscular or oral administrations. The dosage and method of administration vary according to the body-weight and age of a patient and the administration method; however, one skilled in the art can routinely select a suitable metod of administration. If said compound is encodable by a DNA, the DNA can be inserted into a vector for gene therapy and the vector administered to a patient to perform the therapy. The dosage and method of administration vary according to the body-weight, age, and symptoms of the patient but one skilled in the art can suitably select them.

For example, although the dose of a compound that binds to the protein of the present invention and regulates its activity depends on the symptoms, the dose is about 0.1 mg to about 100 mg per day, preferably about 1.0 mg to about 50 mg per day and more preferably about 1.0 mg to about 20 mg per day, when administered orally to a normal adult (weight 60 kg).

When administering parenterally, in the form of an injection to a normal adult (weight 60 kg), although there are some differences according to the patient, target organ, symptoms and method of administration, it is convenient to intravenously inject a dose of about 0.01 mg to about 30 mg per day, preferably about 0.1 to about 20 mg per day and more preferably about 0.1 to about 10 mg per day. Also, in the case of other animals too, it is possible to administer an amount converted to 60 kgs of body-weight.

Assessing the prognosis of a subject with TS

Also provided is a method of assessing the prognosis of a subject with TS by comparing the expression of one or more TS sequences in a test cell population to the expression of the

sequences in a reference cell population derived from patients over a spectrum of disease stages. By comparing gene expression of one or more TS sequences in the test cell population and the reference cell population(s), or by comparing the pattern of gene expression over time in test cell populations derived from the subject, the prognosis of the subject can be assessed.

A decrease in expression of one or more of the sequences TS 347-939 compared to a normal control or an increase of expression of one or more of the sequences TS 1-346 compared to a normal control indicates less favorable prognosis. An increase in expression of one or more of the sequences TS 347-939 indicates a more favorable prognosis, and a decrease in expression of sequences TS 1-346 indicates a more favorable prognosis for the subject.

Kits

The invention also includes a TS-detection reagent, e.g., a nucleic acid that specifically binds to or identifies one or more TS nucleic acids such as oligonucleotide sequences, which are complementary to a portion of a TS nucleic acid or antibodies which bind to proteins encoded by a TS nucleic acid. The reagents are packaged together in the form of a kit. The reagents are packaged in separate containers, e.g., a nucleic acid or antibody (either bound to a solid matrix or packaged separately with reagents for binding them to the matrix), a control reagent (positive and/or negative), and/or a detectable label. Instructions (e.g., written, tape, VCR, CD-ROM, etc.) for carrying out the assay are included in the kit. The assay format of the kit is a Northern hybridization or a sandwich ELISA known in the art.

For example, TS detection reagent is immobilized on a solid matrix such as a porous strip to form at least one TS detection site. The measurement or detection region of the porous strip may include a plurality of sites containing a nucleic acid. A test strip may also contain sites for negative and/or positive controls. Alternatively, control sites are located on a separate strip from the test strip. Optionally, the different detection sites may contain different amounts of immobilized nucleic acids, *i.e.*, a higher amount in the first detection site and lesser amounts in subsequent sites. Upon the addition of test sample, the number of sites displaying a detectable signal provides a quantitative indication of the amount of TS present in the sample. The detection sites may be configured in any suitably detectable shape and are typically in the shape of a bar or dot spanning the width of a teststrip.

Alternatively, the kit contains a nucleic acid substrate array comprising one or more nucleic acid sequences. The nucleic acids on the array specifically identify one or more nucleic acid sequences represented by TS 1-939. The expression of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25,

40 or 50 or more of the sequences represented by TS 1-939 are identified by virtue if the level of binding to an array test strip or chip. The substrate array can be on, *e.g.*, a solid substrate, *e.g.*, a "chip" as described in U.S. Patent No.5,744,305.

Arrays and pluralities

The invention also includes a nucleic acid substrate array comprising one or more nucleic acid sequences. The nucleic acids on the array specifically correspond to one or more nucleic acid sequences represented by TS 1-939. The level expression of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 40 or 50 or more of the sequences represented by TS 1-939 are identified by detecting nucleic acid binding to the array.

The invention also includes an isolated plurality (*i.e.*, a mixture if two or more nucleic acids) of nucleic acid sequences. The nucleic acid sequence are in a liquid phase or a solid phase, *e.g.*, immobilized on a solid support such as a nitrocellulose membrane. The plurality includes one or more of the nucleic acid sequences represented by TS 1-939. In various embodiments, the plurality includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 40 or 50 or more of the sequences represented by TS 1-939.

Methods of inhibiting TS

The invention provides a method for treating or alleviating a symptom of TS in a subject by decreasing expression or activity of TS 1-346 or increasing expression or activity of TS 347-939. Therapeutic compounds are administered prophylactically or therapeutically to subject suffering from at risk of (or susceptible to) developing TS. Such subjects are identified using standard clinical methods or by detecting an aberrant level of expression or activity of (*e.g.*, TS 1-939). Therapeutic agents include inhibitors of cell cycle regulation, cell proliferation, and protein kinase activity.

The therapeutic method includes increasing the expression, or function, or both of one or more gene products of genes whose expression is decreased ("under-expressed genes") in a TS cell relative to normal cells of the same tissue type from which the TS cells are derived. In these methods, the subject is treated with an effective amount of a compound, which increases the amount of one of more of the under-expressed genes in the subject. Administration can be systemic or local. Therapeutic compounds include a polypeptide product of an under-expressed gene, or a biologically active fragment thereof a nucleic acid encoding an under-expressed gene and having expression control elements permitting expression in the TS cells; for example an

agent which increases the level of expression of such gene endogenous to the TS cells (i.e., which up-regulates expression of the under-expressed gene or genes). Administration of such compounds counter the effects of aberrantly-under expressed of the gene or genes in the subjects testis cells and improves the clinical condition of the subject.

The method also includes decreasing the expression, or function, or both, of one or more gene products of genes whose expression is aberrantly increased ("over-expressed gene") in testis cells. Expression is inhibited in any of several ways known in the art. For example, expression is inhibited by administering to the subject a nucleic acid that inhibits, or antagonizes, the expression of the over-expressed gene or genes, e.g., an antisense oligonucleotide or small interfering RNA which disrupts expression of the over-expressed gene or genes.

As noted above, antisense nucleic acids corresponding to the nucleotide sequence of TS 1-346 can be used to reduce the expression level of the TS 1-346. Antisense nucleic acids corresponding to TS 1-346 that are up-regulated in TS are useful for the treatment of TS. Specifically, the antisense nucleic acids of the present invention may act by binding to the TS 1-346 or mRNAs corresponding thereto, thereby inhibiting the transcription or translation of the genes, promoting the degradation of the mRNAs, and/or inhibiting the expression of proteins encoded by the TS 1-346, finally inhibiting the function of the proteins . The term "antisense nucleic acids" as used herein encompasses both nucleotides that are entirely complementary to the target sequence and those having a mismatch of one or more nucleotides, so long as the antisense nucleic acids can specifically hybridize to the target sequences. For example, the antisense nucleic acids of the present invention include polynucleotides that have a homology of at least 70% or higher, preferably at 80% or higher, more preferably 90% or higher, even more preferably 95% or higher over a span of at least 15 continuous nucleotides. Algorithms known in the art can be used to determine the homology.

The antisense nucleic acid derivatives of the present invention act on cells producing the proteins encoded by marker genes by binding to the DNAs or mRNAs encoding the proteins, inhibiting their transcription or translation, promoting the degradation of the mRNAs, and inhibiting the expression of the proteins, thereby resulting in the inhibition of the protein function.

An antisense nucleic acid derivative of the present invention can be made into an external preparation, such as a liniment or a poultice, by mixing with a suitable base material which is inactive against the derivative.

Also, as needed, the derivatives can be formulated into tablets, powders, granules,

capsules, liposome capsules, injections, solutions, nose-drops and freeze-drying agents by adding excipients, isotonic agents, solubilizers, stabilizers, preservatives, pain-killers, and such. These can be prepared by following known methods.

The antisense nucleic acids derivative is given to the patient by directly applying onto the ailing site or by injecting into a blood vessel so that it will reach the site of ailment. An antisense-mounting medium can also be used to increase durability and membrane-permeability. Examples are, liposomes, poly-L-lysine, lipids, cholesterol, lipofectin or derivatives of these.

The dosage of the antisense nucleic acid derivative of the present invention can be adjusted suitably according to the patient's condition and used in desired amounts. For example, a dose range of 0.1 to 100 mg/kg, preferably 0.1 to 50 mg/kg can be administered.

The antisense nucleic acids of the invention inhibit the expression of the protein of the invention and is thereby useful for suppressing the biological activity of a protein of the invention. Also, expression-inhibitors, comprising the antisense nucleic acids of the invention, are useful since they can inhibit the biological activity of a protein of the invention.

The antisense nucleic acids of present invention include modified oligonucleotides. For example, thioated nucleotides may be used to confer nuclease resistance to an oligonucleotide.

Also, a siRNA against marker gene can be used to reduce the expression level of the marker gene. By the term "siRNA" is meant a double stranded RNA molecule which prevents translation of a target mRNA. Standard techniques of introducing siRNA into the cell are used, including those in which DNA is a template from which RNA is transcribed. In the context of the present invention, the siRNA comprises a sense nucleic acid sequence and an anti-sense nucleic acid sequence against an upregulated marker gene, such as TS 1-346. The siRNA is constructed such that a single transcript has both the sense and complementary antisense sequences from the target gene, e.g., a hairpin.

The method is used to alter the expression in a cell of an upregulated, e.g., as a result of malignant transformation of the cells. Binding of the siRNA to a transcript corresponding to one of the TS 1-346 in the target cell results in a reduction in the protein production by the cell. The length of the oligonucleotide is at least 10 nucleotides and may be as long as the naturally-occurring the transcript. Preferably, the oligonucleotide is 19-25 nucleotides in length. Most preferably, the oligonucleotide is less than 75, 50 , 25 nucleotides in length. For example, siRNAs for PYPAF3 comprising nucleotide sequence of SEQ ID NO: 85 or 86 as the target sequence inhibit the cell proliferation of TS.

The nucleotide sequence of the siRNAs were designed using a siRNA design computer

program available from the Ambion website (http://www.ambion.com/techlib/misc/siRNA_finder.html). The computer program selects nucleotide sequences for siRNA synthesis based on the following protocol.

Selection of siRNA Target Sites:

1. Beginning with the AUG start codon of the object transcript, scan downstream for AA dinucleotide sequences. Record the occurrence of each AA and the 3' adjacent 19 nucleotides as potential siRNA target sites. Tuschl, et al. recommend against designing siRNA to the 5' and 3' untranslated regions (UTRs) and regions near the start codon (within 75 bases) as these may be richer in regulatory protein binding sites. UTR-binding proteins and/or translation initiation complexes may interfere with the binding of the siRNA endonuclease complex.
2. Compare the potential target sites to the human genome database and eliminate from consideration any target sequences with significant homology to other coding sequences. The homology search can be performed using BLAST, which can be found on the NCBI server at: www.ncbi.nlm.nih.gov/BLAST/
3. Select qualifying target sequences for synthesis. At Ambion, preferably several target sequences can be selected along the length of the gene for evaluation

The antisense oligonucleotide or siRNA of the invention inhibit the expression of the polypeptide of the invention and is thereby useful for suppressing the biological activity of the polypeptide of the invention. Also, expression-inhibitors, comprising the antisense oligonucleotide or siRNA of the invention, are useful in the point that they can inhibit the biological activity of the polypeptide of the invention. Therefore, a composition comprising the antisense oligonucleotide or siRNA of the present invention are useful in treating a TS.

Alternatively, function of one or more gene products of the over-expressed genes is inhibited by administering a compound that binds to or otherwise inhibits the function of the gene products. For example, the compound is an antibody which binds to the over-expressed gene product or gene products.

The present invention refers to the use of antibodies, particularly antibodies against a protein encoded by an up-regulated marker gene, or a fragment of the antibody. As used herein, the term "antibody" refers to an immunoglobulin molecule having a specific structure, that interacts (i.e., binds) only with the antigen that was used for synthesizing the antibody (i.e., the up-regulated marker gene product) or with an antigen closely related to it. Furthermore, an antibody may be a fragment of an antibody or a modified antibody, so long as it binds to one or

more of the proteins encoded by the marker genes. For instance, the antibody fragment may be Fab, $F(ab')_2$, Fv, or single chain Fv (scFv), in which Fv fragments from H and L chains are ligated by an appropriate linker (Huston J. S. et al. Proc. Natl. Acad. Sci. U.S.A. 85:5879-5883 (1988)). More specifically, an antibody fragment may be generated by treating an antibody with an enzyme, such as papain or pepsin. Alternatively, a gene encoding the antibody fragment may be constructed, inserted into an expression vector, and expressed in an appropriate host cell (see, for example, Co M. S. et al. J. Immunol. 152:2968-2976 (1994); Better M. and Horwitz A. H. Methods Enzymol. 178:476-496 (1989); Pluckthun A. and Skerra A. Methods Enzymol. 178:497-515 (1989); Lamoyi E. Methods Enzymol. 121:652-663 (1986); Rousseaux J. et al. Methods Enzymol. 121:663-669 (1986); Bird R. E. and Walker B. W. Trends Biotechnol. 9:132-137 (1991)).

An antibody may be modified by conjugation with a variety of molecules, such as polyethylene glycol (PEG). The present invention provides such modified antibodies. The modified antibody can be obtained by chemically modifying an antibody. These modification methods are conventional in the field.

Alternatively, an antibody may be obtained as a chimeric antibody, between a variable region derived from a nonhuman antibody and a constant region derived from a human antibody, or as a humanized antibody, comprising the complementarity determining region (CDR) derived from a nonhuman antibody, the frame work region (FR) derived from a human antibody, and the constant region. Such antibodies can be prepared by using known technologies.

Cancer therapies directed at specific molecular alterations that occur in cancer cells have been validated through clinical development and regulatory approval of anti-cancer drugs such as trastuzumab (Herceptin) for the treatment of advanced breast cancer, imatinib methylate (Gleevec) for chronic myeloid leukemia, gefitinib (Iressa) for non-small cell lung cancer (NSCLC), and rituximab (anti-CD20 mAb) for B-cell lymphoma and mantle cell lymphoma (Ciardiello F, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. Clin Cancer Res. 2001 Oct;7(10):2958-70. Review.; Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med. 2001 Mar 15;344(11):783-92.; Rehwald U, Schulz H, Reiser M, Sieber M, Staak JO, Morschhauser F, Driessen C, Rudiger T, Muller-Hermelink K, Diehl V, Engert A. Treatment of relapsed CD20+ Hodgkin lymphoma with the monoclonal antibody rituximab is effective and well tolerated: results of a phase 2 trial

of the German Hodgkin Lymphoma Study Group. Blood. 2003 Jan 15;101(2):420-424.; Fang G, Kim CN, Perkins CL, Ramadevi N, Winton E, Wittmann S and Bhalla KN. (2000). Blood, 96, 2246-2253.). These drugs are clinically effective and better tolerated than traditional anti-cancer agents because they target only transformed cells. Hence, such drugs not only improve survival and quality of life for cancer patients, but also validate the concept of molecularly targeted cancer therapy. Furthermore, targeted drugs can enhance the efficacy of standard chemotherapy when used in combination with it (Gianni L. (2002). Oncology, 63 Suppl 1, 47-56.; Klejman A, Rushen L, Morrione A, Slupianek A and Skorski T. (2002). Oncogene, 21, 5868-5876.). Therefore, future cancer treatments will probably involve combining conventional drugs with target-specific agents aimed at different characteristics of tumor cells such as angiogenesis and invasiveness.

These modulatory methods are performed *ex vivo* or *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). The method involves administering a protein or combination of proteins or a nucleic acid molecule or combination of nucleic acid, molecules as therapy to counteract aberrant expression or activity of the differentially expressed genes.

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity of the genes may be treated with therapeutics that antagonize (*i.e.*, reduce or inhibit) activity of the over-expressed gene or genes. Therapeutics that antagonize activity are administered therapeutically or prophylactically.

Therapeutics that may be utilized include, *e.g.*, (*i*) a polypeptide, or analogs, derivatives, fragments or homologs thereof of the underexpressed sequence or sequences; (*ii*) antibodies to the overexpressed sequence or sequences; (*iii*) nucleic acids encoding the underexpressed sequence or sequences; (*iv*) antisense nucleic acids or nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of one or more overexpressed sequences); (*v*) small interfering RNA (siRNA); or (*vi*) modulators (*i.e.*, inhibitors, agonists and antagonists that alter the interaction between an over/underexpressed polypeptide and its binding partner. The dysfunctional antisense molecules are utilized to "knockout" endogenous function of a polypeptide by homologous recombination (see, *e.g.*, Capecchi, *Science* 244: 1288-1292 1989) .

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with

therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that up-regulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, a polypeptide (or analogs, derivatives, fragments or homologs thereof) or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of a gene whose expression is altered). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, etc.).

Prophylactic administration occurs prior to the manifestation of overt clinical symptoms of disease, such that a disease or disorder is prevented or, alternatively, delayed in its progression.

Therapeutic methods include contacting a cell with an agent that modulates one or more of the activities of the gene products of the differentially expressed genes. An agent that modulates protein activity includes a nucleic acid or a protein, a naturally-occurring cognate ligand of these proteins, a peptide, a peptidomimetic, or other small molecule. For example, the agent stimulates one or more protein activities of one or more of a differentially under-expressed gene.

The present invention also relates to a method of treating or preventing TS in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by a nucleic acid selected from the group consisting of TS 1-346 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide or the fragment thereof. An administration of the polypeptide induce an anti-tumor immunity in a subject. To inducing anti-tumor immunity, a polypeptide encoded by a nucleic acid selected from the group consisting of TS 1-346 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide is administered. The polypeptide or the immunologically active fragments thereof are useful as vaccines against TS. In some cases the proteins or fragments thereof may be administered in a form bound to the T cell recepor (TCR) or presented by an antigen presenting cell (APC), such as macrophage, dendritic cell (DC), or B-cells. Due to the strong antigen presenting ability of DC, the use of DC is most preferable among the APCs.

In the present invention, vaccine against TS refers to a substance that has the function to induce anti-tumor immunity upon inoculation into animals. According to the present invention, polypeptides encoded by TS 1-346 or fragments thereof were suggested to be HLA-A24 or HLA-A*0201 restricted epitopes peptides that may induce potent and specific immune response against TS cells expressing TS 1-346. Thus, the present invention also encompasses method of inducing anti-tumor immunity using the polypeptides. In general, anti-tumor immunity includes immune responses such as follows:

- induction of cytotoxic lymphocytes against tumors,
- induction of antibodies that recognize tumors, and
- induction of anti-tumor cytokine production.

Therefore, when a certain protein induces any one of these immune responses upon inoculation into an animal, the protein is decided to have anti-tumor immunity inducing effect. The induction of the anti-tumor immunity by a protein can be detected by observing in vivo or in vitro the response of the immune system in the host against the protein.

For example, a method for detecting the induction of cytotoxic T lymphocytes is well known. A foreign substance that enters the living body is presented to T cells and B cells by the action of antigen presenting cells (APCs). T cells that respond to the antigen presented by APC in antigen specific manner differentiate into cytotoxic T cells (or cytotoxic T lymphocytes; CTLs) due to stimulation by the antigen, and then proliferate (this is referred to as activation of T cells). Therefore, CTL induction by a certain peptide can be evaluated by presenting the peptide to T cell by APC, and detecting the induction of CTL. Furthermore, APC has the effect of activating CD4+ T cells, CD8+ T cells, macrophages, eosinophils, and NK cells. Since CD4+ T cells and CD8+ T cells are also important in anti-tumor immunity, the anti-tumor immunity inducing action of the peptide can be evaluated using the activation effect of these cells as indicators.

A method for evaluating the inducing action of CTL using dendritic cells (DCs) as APC is well known in the art. DC is a representative APC having the strongest CTL inducing action among APCs. In this method, the test polypeptide is initially contacted with DC, and then this DC is contacted with T cells. Detection of T cells having cytotoxic effects against the cells of interest after the contact with DC shows that the test polypeptide has an activity of inducing the cytotoxic T cells. Activity of CTL against tumors can be detected, for example, using the lysis of ^{51}Cr-labeled tumor cells as the indicator. Alternatively, the method of evaluating the degree of tumor cell damage using ^{3}H-thymidine uptake activity or LDH (lactose dehydrogenase)-

release as the indicator is also well known.

Apart from DC, peripheral blood mononuclear cells (PBMCs) may also be used as the APC. The induction of CTL is reported that the it can be enhanced by culturing PBMC in the presence of GM-CSF and IL-4. Similarly, CTL has been shown to be induced by culturing PBMC in the presence of keyhole limpet hemocyanin (KLH) and IL-7.

The test polypeptides confirmed to possess CTL inducing activity by these methods are polypeptides having DC activation effect and subsequent CTL inducing activity. Therefore, polypeptides that induce CTL against tumor cells are useful as vaccines against tumors. Furthermore, APC that acquired the ability to induce CTL against tumors by contacting with the polypeptides are useful as vaccines against tumors. Furthermore, CTL that acquired cytotoxicity due to presentation of the polypeptide antigens by APC can be also used as vaccines against tumors. Such therapeutic methods for tumors using anti-tumor immunity due to APC and CTL are referred to as cellular immunotherapy.

Generally, when using a polypeptide for cellular immunotherapy, efficiency of the CTL-induction is known to increase by combining a plurality of polypeptides having different structures and contacting them with DC. Therefore, when stimulating DC with protein fragments, it is advantageous to use a mixture of multiple types of fragments.

Alternatively, the induction of anti-tumor immunity by a polypeptide can be confirmed by observing the induction of antibody production against tumors. For example, when antibodies against a polypeptide are induced in a laboratory animal immunized with the polypeptide, and when growth of tumor cells is suppressed by those antibodies, the polypeptide can be determined to have an ability to induce anti-tumor immunity.

Anti-tumor immunity is induced by administering the vaccine of this invention, and the induction of anti-tumor immunity enables treatment and prevention of TS. Therapy against cancer or prevention of the onset of cancer includes any of the steps, such as inhibition of the growth of cancerous cells, involution of cancer, and suppression of occurrence of cancer. Decrease in mortality of individuals having cancer, decrease of tumor markers in the blood, alleviation of detectable symptoms accompanying cancer, and such are also included in the therapy or prevention of cancer. Such therapeutic and preventive effects are preferably statistically significant. For example, in observation, at a significance level of 5% or less, wherein the therapeutic or preventive effect of a vaccine against cell proliferative diseases is compared to a control without vaccine administration. For example, Student's t-test, the Mann-Whitney U-test, or ANOVA may be used for statistical analyses.

The above-mentioned protein having immunological activity or a vector encoding the protein may be combined with an adjuvant. An adjuvant refers to a compound that enhances the immune response against the protein when administered together (or successively) with the protein having immunological activity. Examples of adjuvants include cholera toxin, salmonella toxin, alum, and such, but are not limited thereto. Furthermore, the vaccine of this invention may be combined appropriately with a pharmaceutically acceptable carrier. Examples of such carriers are sterilized water, physiological saline, phosphate buffer, culture fluid, and such. Furthermore, the vaccine may contain as necessary, stabilizers, suspensions, preservatives, surfactants, and such. The vaccine is administered systemically or locally. Vaccine administration may be performed by single administration, or boosted by multiple administrations.

When using APC or CTL as the vaccine of this invention, tumors can be treated or prevented, for example, by the ex vivo method. More specifically, PBMCs of the subject receiving treatment or prevention are collected, the cells are contacted with the polypeptide ex vivo, and following the induction of APC or CTL, the cells may be administered to the subject. APC can be also induced by introducing a vector encoding the polypeptide into PBMCs ex vivo. APC or CTL induced in vitro can be cloned prior to administration. By cloning and growing cells having high activity of damaging target cells, cellular immunotherapy can be performed more effectively. Furthermore, APC and CTL isolated in this manner may be used for cellular immunotherapy not only against individuals from whom the cells are derived, but also against similar types of tumors from other individuals.

Furthermore, a pharmaceutical composition for treating or preventing a cell proliferative disease, such as cancer, comprising a pharmaceutically effective amount of the polypeptide of the present invention is provided. The pharmaceutical composition may be used for raising anti tumor immunity.

Pharmaceutical compositions for inhibiting TS

Pharmaceutical formulations include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration, or for administration by inhalation or insufflation. Preferably, administration is intravenous. The formulations are optionally packaged in discrete dosage units.

Pharmaceutical formulations suitable for oral administration include capsules, cachets or tablets, each containing a predetermined amount of the active ingredient. Formulations also include powders, granules or solutions, suspensions or emulsions. The active ingredient os optionally administered as a bolus electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrant or wetting agents. A tablet may be made by compression or molding, optionally with one or more formulational ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be coated according to methods well known in the art. Oral fluid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives. The tablets may optionally be formulated so as to provide slow or controlled release of the active ingredient therein. A package of tablets may contain one tablet to be taken on ech of the month.

Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline, water-for-injection, immediately prior to use. Alternatively, the formulations may be presented for continuous infusion. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration include suppositories with standard carriers such as cocoa butter or polyethylene glycol. Formulations for topical administration in the mouth, for example buccally or sublingually, include lozenges, which contain the active ingredient in a flavored base such as sucrose and acacia or tragacanth, and pastilles comprising the active ingredient in a base such as gelatin and glycerin or sucrose and acacia. For intra-nasal

administration the compounds of the invention may be used as a liquid spray or dispersible powder or in the form of drops. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents.

For administration by inhalation the compounds are conveniently delivered from an insufflator, nebulizer, pressurized packs or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichiorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, the compounds may take the form of a dry powder composition, for example a powder mix of the compound and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form, in for example, capsules, cartridges, gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflators.

Other formulations include implantable devices and adhesive patches; which release a therapeutic agent.

When desired, the above described formulations, adapted to give sustained release of the active ingredient, may be employed. The pharmaceutical compositions may also contain other active ingredients such as antimicrobial agents, immunosuppressants or preservatives.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include flavoring agents.

Preferred unit dosage formulations are those containing an effective dose, as recited below, or an appropriate fraction thereof, of the active ingredient.

For each of the aforementioned conditions, the compositions, e.g., polypeptides and organic compounds are administered orally or via injection at a dose of from about 0.1 to about 250 mg/kg per day. The dose range for adult humans is generally from about 5 mg to about 17.5 g/day, preferably about 5 mg to about 10 g/day, and most preferably about 100 mg to about 3 g/day. Tablets or other unit dosage forms of presentation provided in discrete units may conveniently contain an amount which is effective at such dosage or as a multiple of the same, for instance, units containing about 5 mg to about 500 mg, usually from about 100 mg to about 500 mg.

The dose employed will depend upon a number of factors, including the age and sex of the subject, the precise disorder being treated, and its severity. Also the route of administration may vary depending upon the condition and its severity.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims. The following examples illustrate the identification and characterization of genes differentially expressed in TS cells.

EXAMPLE 1: PREPARATION OF TEST SAMPLES

Tissue obtained from diseased tissue (e.g., testis cells from testicular germ cell tumors) and normal tissues were evaluated to identify genes which are differently expressed or a disease state, *e.g.*, TS. The assays were carried out as follows.

Patients, tissue samples, and laser-capture microdissection (LCM)

TGCT samples were obtained from 13 patients who underwent orchiectomy. Clinical features of these patients are summarized in Table 1. 12 samples diagnosed as seminoma and one sample of both seminoma and yolk sac tumor were used.

All samples were frozen at -80^{O}C and then embedded in TissueTek OCT medium (Sakura). The frozen specimens were serially sectioned in 8-μm slices with cryostat (Sakura) and were stained with hematoxylin and eosin to define the analyzed regions. Then, seminoma cells were selectively microdissected from each stained tissue with the PixCell II LCM System (Arcturus Engineering) following the manufacture's protocol with several modifications (21).

Table1. Clinical characteristics of thirteen testicular seminomas

Case No.	Age	Histopathological type	Stage	Outcome
1	43	seminoma	I	survival
2	20	seminoma	I	survival
3	34	seminoma	I	survival
4	33	seminoma	I	survival
5	26	seminoma	I	survival
6	34	seminoma	I	survival
7	45	seminoma	I	survival
8	24	seminoma	I	survival
9	44	seminoma	I	survival
10	27	seminoma	I	survival
11	49	seminoma	I	survival
12	42	seminoma	III B	survival
13	33	seminoma + yolk sac tumor	II B	survival

Extraction and purification of RNA and T7-based RNA amplification

Total RNAs were extracted from captured cells into 350μl RLT lysis buffer (QIAGEN).

The extracted RNAs were treated for 15 minutes at room temperature with 30 units of DNase I (QIAGEN). All of the DNase I treated RNAs were subjected to T7-based amplification using Ampliscribe T7 Transcription Kit (Epicentre Technologies)(20). Two rounds of amplification yielded 30 - 238μg of amplified RNA (aRNA) for each tissue. As the control probe, normal human poly(A)$^+$ RNA (Clontech) was amplified two rounds by the T7-based amplification. 2.5μg aliquots of aRNAs from each cancerous tissue and the control were reverse-transcribed in the presence of Cy5-dCTP and Cy3-dCTP, respectively (22).

Preparation of the cDNA microarray

A "genome-wide" cDNA microarray system was established containing 23,040 cDNAs selected from the UniGene database (build #131) of the National Center for Biotechnology Information (NCBI). Briefly, the cDNAs were amplified by RT-PCR using poly (A)$^+$ RNA isolated from various human organs as templates; the lengths of the amplicons ranged from 200 to 1,100 bp excluding repetitive or poly(A) sequences. The PCR products were spotted on type 7 glass slides using a Microarray Spotter, Generation III (Amersham Biosciences); 4608 genes were spotted in duplicate on a single slide. Five different sets of slides were prepared (i.e., 23,040 genes total), on each of which the same 52 housekeeping genes and two negative-control genes were spotted as well (23).

Hybridization and acquisition of data

Hybridization and washing were performed according to protocols described previously except that all processes were carried out with an Automated Slide Processor (Amersham Biosciences). The intensity of each hybridization signal was calculated photometrically by the ArrayVision computer program (Amersham Biosciences) and background intensity was subtracted. Normalization of each Cy3- and Cy5-signal intensity was performed using averaged signals from the 52 housekeeping genes. A cut-off value for each expression level was automatically calculated according to background fluctuation. Cy5/Cy3 was calculated as the relative expression ratio. When both Cy3 and Cy5 signal intensities were lower than the cut-off values, expression of the corresponding gene in that sample was assessed as absent according to previous report (23). For other genes the Cy5/Cy3 ratio was calculated using raw data of each sample.

EXAMPLE 2: IDENTIFICATION OF TS – ASSOCIATED GENES

When up- or down-regulated genes common to TS were identified, the genes were analyzed according to the following criteria. Initially genes were selected whose relative expression ratio was able to calculate of more than 50% cases and whose expression were up- or down-regulated in more than 70% of cases. Moreover, if the relative expression ratio was able to calculate of 35 to 50% cases, the genes were also evaluated that all of cases were up- or down-regulated. The relative expression ratio of each gene (Cy5/Cy3 intensity ratio) was classified into one of four categories as follows: (1) up-regulated (expression ratio was more than 5.0); (2) down-regulated (expression ratio less than 0.2); (3) unchanged expression (expression ratio between 0.2 and 5.0); and (4) not expressed (or slight expression but under the cut-off level for detection). These categories were used to detect a set of genes whose changes in expression ratios were common among samples as well as specific to a certain subgroup. To detect candidate genes that were commonly up- or down- regulated in seminoma cells, the overall expression patterns of 23,040 genes were screened to select genes with expression ratios of more than 5.0 or less than 0.2.

Identification of genes with clinically relevant expression patterns in TS cells

To elucidate genetic events underlying development and progression of TGCTs, we analyzed gene expression in clinical materials by means of a genome-wide cDNA microarray. Microarray technology makes it possible to analyze expression of thousands of genes in a single experiment, and to gain new insights into molecular mechanisms of cancer. Such data are expected to contribute to improvement of clinical management and thereby provide a better quality of life for cancer patients.

One group of investigators analyzed gene-expression profiles using a custom-made cDNA microarray of genes located on chromosome 17 (13), because the long arm of chromosome 17 is frequently over-represented in TGCTs. However, only 636 genes on chromosome 17 and 512 genes from elsewhere in the genome were analyzed in that study. To our knowledge ours is the first "genome-wide" cDNA microarray analysis of TGCTs.

We focused especially on TS, using a comprehensive cDNA microarray system containing 23,040 genes to examine populations of seminoma cells purified by LCM. The proportion of cancer cells selected by this procedure was estimated to be nearly 100%, as determined by microscpic visualization.

Three hundred forty-six up-regulated genes whose expression ratio was more than 5.0 were identified (Table 3), whereas 593 down-regulated genes whose expression ratio was less

than 0.2 were identified (Table 4). Furthermore, in particular, 213 highly up-regulated genes whose expression ratio was more than 10.0 were identified (data not shown). On the other hand, 376 down-regulated genes whose expression ratio was less than 0.1 were identified (data not shown).

Some of them might represent potential molecular targets for new therapeutic agents, and/or serve as diagnostic tumor markers. The list of genes in Table 3 included CCND2 (1), POV1 (24), PIM2 (25), JUP (26), and MYCN (14), genes already known to be involved in carcinogenesis or cell proliferation of TS. For example CCND2, which regulates the phosphorylation of RB protein and controls the G1-S cell cycle checkpoint, is often highly expressed in TS; disruption of this checkpoint through over-expression of D-type cyclin is one of the major pathways for tumor development in humans (1). POV1, first identified as a gene that was over-expressed in prostate cancers (24), was later shown to be highly expressed in all TS as well as in carcinoma in situ of the testis (13). This gene encodes a membrane-transport protein with 12 transmembrane domains and may transport nutrients and/or metabolites essential to cell growth (27). Therefore, its product might be a potential molecular-target for anti-cancer drugs for treating TS and prostate cancers. PIM2, a proto-oncogene encoding a serine threonine kinase, was previously reported to be highly expressed in hematopoietic stem cells, leukemic and lymphoma cell lines, and TS; its product appears to have a critical role in hematopoiesis and in oncogenic transformation (25). JUP, also known as gamma-catenin, plays an important role in cell adhesion and the Wnt signaling pathway; JUP is regulated by the APC tumor suppressor gene, and its oncogenic activity in colon cancers is thought to be distinct from that of beta-catenin (26). Amplification of the MYCN gene has been observed in a variety of human tumors, most frequently in neuroblastomas, and its over-expression has been documented in both seminomas and non-seminomas (14). Thus, suppression of these oncogenic functions might be a novel approach to treatment of TS. Moreover, these up-regulated elements included significant genes involved in signal transduction pathway, oncogenes, cell cycle, and cell adhesion and cytoskeleton (Table 5).

In addition to genes known to have some involvement in testicular carcinomas, we noted over-expression of other oncogenes including PIM-1, RET and VAV2. PIM-1, encoding a serine/threonine kinase (28), was over-expressed in all of the 11 informative seminomas examined on our microarray. RET was also over-expressed in all of the six informative seminomas. The RET gene encodes a receptor tyrosine kinase, a cell-surface molecule that transduces signals for cell growth and differentiation; germline mutations in the RET gene are

responsible for two hereditary cancer syndromes, multiple endocrine neoplasia types 2A and 2B (29). VAV2, a member of the VAV oncogene family, was over-expressed in 11 of the 12 informative seminoma cases tested on our microarray. The VAV protein is associated with cellular transformation and oncogenesis; it seems to either enhance the metastatic properties of transformed cells or serve as an ancillary factor contributing to the transforming activities of oncoproteins such as Ras (30).

On the other hand, our list of down-regulated genes included at least one known tumor suppressor, WT1, whose inactivation causes Wilms tumor and also WAGR syndrome, which is characterized by susceptibility to Wilms tumor, aniridia, genitourinary abnormalities, and mental retardation (31). Loss of heterozygosity in the chromosomal region harboring WT1 has been observed frequently in testicular germ cell tumors (32). Furthermore, Wilms tumor 1-associating protein (KIAA0105, WTAP), a WT1-binding partner, was also down-regulated in our study. Since WT1 is related to normal development of the genitourinary system, its product may be one a candidate for involvement in testicular carcinogenesis although its molecular mechanism remains unclear.

Recent achievement of clinical improvements through use of molecular-targeted drugs has underscored the importance of discovering new molecular targets for development of drugs to treat specific cancers. For example, an anti-HER2 monoclonal antibody, trastuzumab, in conjunction with anti-cancer drugs, antagonizes the proto-oncogene receptor HER2/neu and leads to improvement of clinical response and survival of some breast-cancer patients (33). STI-571, a tyrosine kinase inhibitor targeting bcr-abl, is now a first-line drug for treatment of chronic myeloid leukemias (34), and an epidermal growth factor receptor inhibitor, gefitinib, is useful for treatment of non-small cell lung cancers (35). An anti-CD20 monoclonal antibody, rituximab, has improved rates of complete remission and overall survival for patients with B-cell lymphoma or mantle cell lymphoma (36). Hence, the up-regulated gene products which were identified here and are related to cell proliferation may be promising potential targets for designing novel agents for treating TS. In particular, secreted proteins that function in the autocrine cell-growth pathway should be good candidates for development of drugs and could become novel diagnostic markers for this type of cancer.

Eleven of the 13 cases analyzed in this study were classified clinicopathologically to stage I. Hence, genes which were commonly up-regulated or down-regulated on our microarray are likely to be associated with relatively early phases of carcinogenesis. Consequently, our data provide not only new information about cancer-related genes but also a

new correlation of known genes with carcinogenesis. Nonetheless, the information described in this paper disclosed a high degree of complexity among alterations in genetic activities during development of TS; the result is a long list of potential therapeutic targets and/or biomarkers for this type of cancer.

5

Table 3 346 genes commomly up-regulated five-fold or more in testicular seminomas.

TS Assignment	Accession No.	Symbol	Gene name
1	AI141839	ABCD4	ATP-binding cassette, sub-family D (ALD), member 4
2	X02994	ADA	adenosine deaminase
3	U41767	ADAM15	a disintegrin and metalloproteinase domain 15 (metargidin)
4	AF024714	AIM2	absent in melanoma 2
5	H57960	AK3	adenylate kinase 3
6	U24266	ALDH4	aldehyde dehydrogenase 4 (glutamate gamma-semialdehyde dehydrogenase; pyrroline-5-carboxylate dehydrogenase)
7	AA180314	ANKRD2	Ankyrin repeat domain 2 (stretch responsive muscle)
8	AA910946	AP1M2	adaptor-related protein complex 1, mu 2 subunit
9	AA676726	APELIN	apelin; peptide ligand for APJ receptor
10	U79268	APEX	APEX nuclease (multifunctional DNA repair enzyme)
11	X00570	APOC1	apolipoprotein C-I
12	L08424	ASCL1	achaete-scute complex (Drosophila) homolog-like 1
13	D89052	ATP6F	ATPase, H+ transporting, lysosomal (vacuolar proton pump) 21kD
14	AF038195	BCS1L	BCS1 (yeast homolog)-like
15	M88714	BDKRB2	bradykinin receptor B2
16	AF001383	BIN1	bridging integrator 1
17	W91908	BRAG	B cell RAG associated protein
18	R43935	CACNA1G	calcium channel, voltage-dependent, alpha 1G subunit
19	U66063	CAMK2G	calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma
20	AA682870	CCND2	cyclin D2
21	U45983	CCR8	chemokine (C-C motif) receptor 8
22	M16445	CD2	CD2 antigen (p50), sheep red blood cell receptor
23	AA083656	CD37	CD37 antigen

24	M37033	CD53	CD53 antigen
25	M81934	CDC25B	cell division cycle 25B
26	X63629	CDH3	cadherin 3, type 1, P-cadherin (placental)
27	M16965	CDR1	cerebellar degeneration-related protein (34kD)
28	U51095	CDX1	caudal type homeo box transcription factor 1
29	AA319695	CEBPD	CCAAT/enhancer binding protein (C/EBP), delta
30	U14518	CENPA	centromere protein A (17kD)
31	U58514	CHI3L2	chitinase 3-like 2
32	X14830	CHRNB1	cholinergic receptor, nicotinic, beta polypeptide 1 (muscle)
33	AC002115	COX6B	cytochrome c oxidase subunit VIb
34	X59932	CSK	c-src tyrosine kinase
35	AW167729	CTSC	cathepsin C
36	AA579959	CYP2S1	cytochrome P540 family member predicted from ESTs
37	N20321	D19S1177E	DNA segment on chromosome 19 (unique) 1177 expressed sequence
38	U79775	D21S2056E	DNA segment on chromosome 21 (unique) 2056 expressed sequence
39	AI092999	D2S448	Melanoma associated gene
40	Z29093	DDR1	discoidin domain receptor family, member 1
41	U49785	DDT	D-dopachrome tautomerase
42	T78186	DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha
43	D78011	DPYS	dihydropyrimidinase
44	U88047	DRIL1	dead ringer (Drosophila)-like 1
45	AA128470	DSP	desmoplakin (DPI, DPII)
46	X92896	DXS9879E	DNA segment on chromosome X (unique) 9879 expressed sequence
47	AA233853	E1B-AP5	E1B-55kDa-associated protein 5
48	S49592	E2F1	E2F transcription factor 1
49	AA422074	ENO2	Enolase 2, (gamma, neuronal)
50	M57736	ENPP1	ectonucleotide pyrophosphatase/phosphodiesterase1
51	U07695	EPHB4	EphB4
52	U15655	ERF	Ets2 repressor factor
53	D12765	ETV4	ets variant gene 4 (E1A enhancer-binding protein, E1AF)
54	X86779	FASTK	Fas-activated serine/threonine kinase
55	J04162	FCGR3B	Fc fragment of IgG, low affinity IIIb, receptor for (CD16)
56	M60922	FLOT2	flotillin 2
57	R72881	GABBR1	gamma-aminobutyric acid (GABA)

			B receptor, 1
58	AF077740	GCAT	glycine C-acetyltransferase (2-amino-3-ketobutyrate coenzyme A ligase)
59	M18185	GIP	gastric inhibitory polypeptide
60	AA669536	GJA5	Gap junction protein, alpha 5, 40kD (connexin 40)
61	U78027	GLA	galactosidase, alpha
62	N26076	GOV	glioblastoma overexpressed
63	D64154	GP110	cell membrane glycoprotein, 110000M(r) (surface antigen)
64	AF062006	GPR49	G protein-coupled receptor 49
65	AA877534	GPRC5C	G protein-coupled receptor, family C, group 5, member C
66	X68314	GPX2	glutathione peroxidase 2 (gastrointestinal)
67	AI346758	GYG2	glycogenin 2
68	J04501	GYS1	glycogen synthase 1 (muscle)
69	U26174	GZMK	granzyme K (serine protease, granzyme 3; tryptase II)
70	X57129	H1F2	H1 histone family, member 2
71	AA904505	H3FD	H3 histone family, member D
72	M16707	H4F2	H4 histone, family 2
73	M58285	HEM1	hematopoietic protein 1
74	AA903016	HM74	putative chemokine receptor; GTP-binding protein
75	D66904	HRMT1L2	HMT1 (hnRNP methyltransferase, S. cerevisiae)-like 2
76	AW084318	HSPB1	heat shock 27kD protein 1
77	AA564686	HSPC025	HSPC025
78	AA775500	HsPOX2	proline oxidase 2
79	AI189477	IDH2	isocitrate dehydrogenase 2 (NADP+), mitochondrial
80	AA436509	IER5	Immediate early response 5
81	X16302	IGFBP2	insulin-like growth factor binding protein 2 (36kD)
82	AJ001563	IGHG3	immunoglobulin heavy constant gamma 3 (G3m marker)
83	M87790	IGL λ	immunoglobulin lambda locus
84	AI189680	IL1RAP	interleukin 1 receptor accessory protein
85	M20566	IL6R	interleukin 6 receptor
86	J05272	IMPDH1	IMP (inosine monophosphate) dehydrogenase 1
87	S78296	INA	internexin neuronal intermediate filament protein, alpha
88	M15395	ITGB2	integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associated antigen 1; macrophage

			antigen 1 (mac) beta subunit)
89	X16260	ITIH1	inter-alpha (globulin) inhibitor, H1 polypeptide
90	AA226073	ITM2C	integral membrane protein 2C
91	AI205103	ITPK1	inositol 1,3,4-triphosphate 5/6 kinase
92	Z68228	JUP	junction plakoglobin
93	AA707252	KIAA0468	Syndecan 3 (N-syndecan)
94	D52745	KIAA0821	lectomedin-2
95	H06478	KIF3C	kinesin family member 3C
96	U06698	KIF5A	kinesin family member 5A
97	AA845512	KLF4	Kruppel-like factor 4 (gut)
98	X77744	KR18	KRAB zinc finger protein KR18
99	X87342	LLGL2	lethal giant larvae (Drosophila) homolog 2
100	BF971926	LMNA	lamin A/C
101	AI298111	LOC51116	CGI-91 protein
102	AA714315	LOC51181	carbonyl reductase
103	D89078	LTB4R	leukotriene b4 receptor (chemokine receptor-like 1)
104	U42376	LY6E	lymphocyte antigen 6 complex, locus E
105	AC005546	LYL1	lymphoblastic leukemia derived sequence 1
106	AA179832	M6PR	mannose-6-phosphate receptor (cation dependent)
107	D87116	MAP2K3	mitogen-activated protein kinase kinase 3
108	AA583183	MAP4K3	mitogen-activated protein kinase kinase kinase kinase 3
109	AA744607	MASL1	MFH-amplified sequences with leucine-rich tandem repeats 1
110	X74795	MCM5	minichromosome maintenance deficient (S. cerevisiae) 5 (cell division cycle 46)
111	U78313	MDFI	MyoD family inhibitor
112	L10612	MIF	macrophage migration inhibitory factor (glycosylation-inhibiting factor)
113	J05070	MMP9	matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase)
114	H46518	MRPS26	Mitochondrial ribosomal protein S26
115	AA101822	MSDC1	Mesoderm development candidate 1
116	N70019	MT1E	metallothionein 1E (functional)
117	AI094778	MT2A	metallothionein 2A
118	J04031	MTHFD1	methylenetetrahydrofolate dehydrogenase (NADP+ dependent),

			methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthetase
119	X13293	MYBL2	v-myb avian myeloblastosis viral oncogene homolog-like 2
120	Y00664	MYCN	V-myc avian myelocytomatosis viral related oncogene, neuroblastoma derived
121	AI188406	NDUFA4	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4 (9kD, MLRQ)
122	AA989104	NDUFB2	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 2 (8kD, AGGG)
123	X83957	NEB	nebulin
124	H08616	NESCA	nesca protein
125	AA977227	NET-6	tetraspan NET-6 protein
126	W46617	NF2	Neurofibromin 2 (bilateral acoustic neuroma)
127	AI300590	NFE2L3	nuclear factor (erythroid-derived 2)-like 3
128	X77909	NFKBIL1	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 1
129	AJ001258	NIPSNAP1	NIPSNAP, C. elegans, homolog 1
130	U23070	NMA	putative transmembrane protein
131	X17620	NME1	non-metastatic cells 1, protein (NM23A) expressed in
132	L16785	NME2	non-metastatic cells 2, protein (NM23B) expressed in
133	AA242961	NOD1	caspase recruitment domain 4
134	AI085648	NOLA3	nucleolar protein family A, member 3 (H/ACA small nucleolar RNPs)
135	U56079	NPY5R	neuropeptide Y receptor Y5
136	AA628440	NR1I3	nuclear receptor subfamily 1, group I, member 3
137	R16767	NRBP	nuclear receptor binding protein
138	AI049668	OAZ1	ornithine decarboxylase antizyme 1
139	D10523	OGDH	oxoglutarate dehydrogenase (lipoamide)
140	X17094	PACE	paired basic amino acid cleaving enzyme (furin, membrane associated receptor protein)
141	AI146846	PAR3	three-PDZ containing protein similar to C. elegans PAR3 (partitioning defect)
142	AI248183	PAX5	Paired box gene 5 (B-cell lineage specific activator protein)
143	AI265770	PDLIM1	PDZ and LIM domain 1 (elfin)
144	X54936	PGF	placental growth factor, vascular

			endothelial growth factor-related protein
145	AA532444	PHLDA3	pleckstrin homology-like domain, family A, member 3
146	X80907	PIK3R2	phosphoinositide-3-kinase, regulatory subunit, polypeptide 2 (p85 beta)
147	M16750	PIM1	pim oncogene
148	U77735	PIM2	pim-2 oncogene
149	D00244	PLAU	plasminogen activator, urokinase
150	X07743	PLEK	pleckstrin
151	M80397	POLD1	polymerase (DNA directed), delta 1, catalytic subunit (125kD)
152	S90469	POR	P450 (cytochrome) oxidoreductase
153	AF045584	POV1	prostate cancer overexpressed gene 1
154	S57501	PPP1CA	protein phosphatase 1, catalytic subunit, alpha isoform
155	N44532	PPP1R14C	Protein phosphatase 1, regulatory (inhibitor) subunit 14C
156	AI274279	PRDM4	PR domain containing 4
157	AI309741	PRG6	p53-responsive gene 6
158	AF027208	PROML1	prominin (mouse)-like 1
159	M24398	PTMS	parathymosin
160	U47025	PYGB	phosphorylase, glycogen; brain
161	Y15233	PYGL	phosphorylase, glycogen; liver (Hers disease, glycogen storage disease type VI)
162	AA346311	RAI3	retinoic acid induced 3
163	M29893	RALA	v-ral simian leukemia viral oncogene homolog A (ras related)
164	Y00291	RARB	retinoic acid receptor, beta
165	Y12336	RASGRP2	RAS guanyl releasing protein 2 (calcium and DAG-regulated)
166	X64652	RBMS1	RNA binding motif, single stranded interacting protein 1
167	AF040105	RCL	putative c-Myc-responsive
168	AA807607	RDGBB	retinal degeneration B beta
169	AA932768	REPRIMO	candidate mediator of the p53-dependent G2 arrest
170	X12949	RET	ret proto-oncogene (multiple endocrine neoplasia MEN2A, MEN2B and medullary thyroid carcinoma 1, Hirschsprung disease)
171	NM_139176	PYPAF3	PYRIN-containing Apaf-1-like protein 3
172	AA921313	RPL11	ribosomal protein L11
173	L11566	RPL18	ribosomal protein L18
174	AA402920	RPL18A	ribosomal protein L18a

175	AA962580	RPL22	ribosomal protein L22
176	AI123363	RPL23A	ribosomal protein L23a
177	AI341159	RPL26	ribosomal protein L26
178	AA313541	RPL37	ribosomal protein L37
179	R50505	RPLP1	ribosomal protein, large, P1
180	AI131289	RPLP2	ribosomal protein, large P2
181	M81757	RPS19	ribosomal protein S19
182	L04483	RPS21	ribosomal protein S21
183	N27409	RPS23	ribosomal protein S23
184	U14970	RPS5	ribosomal protein S5
185	X99920	S100A13	S100 calcium-binding protein A13
186	AI261620	SAAS	granin-like neuroendocrine peptide precursor
187	U72355	SAFB	scaffold attachment factor B
188	X98834	SALL2	sal (Drosophila)-like 2
189	T30682	SCO2	SCO cytochrome oxidase deficient homolog 2 (yeast)
190	AB000887	SCYA19	small inducible cytokine subfamily A (Cys-Cys), member 19
191	AA534943	SCYB14	small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK)
192	AI080351	SEC63L	SEC63, endoplasmic reticulum translocon component (S. cerevisiae) like
193	K01396	SERPINA1	serine (or cysteine) proteinase inhibitor, clade A (alpha antiproteinase, antitrypsin), member 1
194	AI050752	SGCB	Sarcoglycan, beta (43kD dystrophin-associated glycoprotein)
195	AA421248	SH3BGRL3	SH3 domain binding glutamic acid-rich protein like 3
196	L11932	SHMT1	serine hydroxymethyltransferase 1
197	T29731	SHMT2	serine hydroxymethyltransferase 2 (mitochondrial)
198	U44403	SLA	Src-like-adapter
199	J03592	SLC25A6	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 6
200	AW511361	SLC29A1	solute carrier family 29 (nucleoside transporters), member 1
201	D84454	SLC35A2	solute carrier family 35 (UDP-galactose transporter), member 2
202	M65105	SLC6A2	solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2
203	AW504047	SMARCA4	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4

204	AI143147	SNRPF	small nuclear ribonucleoprotein polypeptide F
205	X70683	SOX4	SRY (sex determining region Y)-box 4
206	U49240	SPK	symplekin; Huntingtin interacting protein I
207	J03161	SRF	serum response factor (c-fos serum response element-binding transcription factor)
208	AA683542	STAU2	staufen (Drosophila, RNA-binding protein) homolog 2
209	AI151087	T1A-2	lung type-I cell membrane-associated glycoprotein
210	AA235074	TCF19	transcription factor 19 (SC1)
211	X82240	TCL1A	T-cell leukemia/lymphoma 1A
212	AA399645	TCOF1	Treacher Collins-Franceschetti syndrome 1
213	U85658	TFAP2C	transcription factor AP-2 gamma (activating enhancer-binding protein 2 gamma)
214	AI049960	TGIF2	TGFB-induced factor 2 (TALE family homeobox)
215	AA293042	THY1	Thy cell surface antigen
216	AJ005895	TIM17B	translocase of inner mitochondrial membrane 17 (yeast) homolog B
217	AA536113	TMEPAI	transmembrane, prostate androgen induced RNA
218	AI261341	TMP21	transmembrane trafficking protein
219	M64247	TNNI3	troponin I, cardiac
220	M19309	TNNT1	troponin T1, skeletal, slow
221	M19713	TPM1	tropomyosin 1 (alpha)
222	AA890188	TUBG2	tubulin, gamma 2
223	AA481924	TYROBP	TYRO protein tyrosine kinase binding protein
224	U73379	UBCH10	ubiquitin carrier protein E2-C
225	AA465240	VAV2	vav 2 oncogene
226	Z71621	WNT2B	wingless-type MMTV integration site family, member 2B
227	AA644644	YWHAH	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide
228	AA555115	LOC51260	hypothetical protein
229	AA056472	LOC57228	hypothetical protein from clone 643
230	R37098	DKFZp547M236	hypothetical protein DKFZp547M236
231	AA776240	DKFZP586J0917	DKFZP586J0917 protein
232	AA609417	DKFZp762M136	hypothetical protein DKFZp762M136

233	N80485	FLJ10199	hypothetical protein FLJ10199
234	W18181	FLJ10430	hypothetical protein FLJ10430
235	U69190	FLJ10432	hypothetical protein
236	AA287875	FLJ10549	hypothetical protein FLJ10549
237	AI206219	FLJ10634	hypothetical protein FLJ10634
238	AA368409	FLJ10688	hypothetical protein FLJ10688
239	AI014673	FLJ10709	hypothetical protein FLJ10709
240	AA219141	FLJ10713	hypothetical protein FLJ10713
241	AA477929	FLJ10767	hypothetical protein FLJ10767
242	AK026707	FLJ11328	hypothetical protein FLJ11328
243	AA306716	FLJ11937	hypothetical protein FLJ11937
244	AI017753	FLJ20069	hypothetical protein FLJ20069
245	AA843844	FLJ20171	hypothetical protein FLJ20171
246	AI360274	FLJ20494	similar to mouse neuronal protein 15.6
247	AI276023	FLJ20539	hypothetical protein FLJ20539
248	AA058761	FLJ20550	hypothetical protein FLJ20550
249	Z24980	FLJ22195	hypothetical protein FLJ22195
250	AA813912	KIAA0130	KIAA0130 gene product
251	AA394063	KIAA0144	KIAA0144 gene product
252	AI090862	KIAA0147	human homolog of Drosophila Scribble
253	AB007925	KIAA0456	KIAA0456 protein
254	AB014544	KIAA0644	KIAA0644 gene product
255	AB014590	KIAA0690	KIAA0690 protein
256	AA954348	KIAA0870	KIAA0870 protein
257	AA737525	KIAA1031	KIAA1031 protein
258	AA443202	KIAA1053	KIAA1053 protein
259	W90578	KIAA1198	KIAA1198 protein
260	AA191449	KIAA1254	KIAA1254 protein
261	AI076459	KIAA1272	Homo sapiens cDNA FLJ12819 fis, clone NT2RP2002727, weakly similar to Rattus norvegicus tulip 2 mRNA
262	AA579859	KIAA1273	KIAA1273 protein
263	AA731891	KIAA1517	KIAA1517 protein
264	AI093595	LOC55895	22kDa peroxisomal membrane protein-like
265	AA149846		Homo sapiens mRNA; cDNA DKFZp762B195 (from clone DKFZp762B195)
266	AA741366		Homo sapiens mRNA; cDNA DKFZp761K2312 (from clone DKFZp761K2312)
267	AA400449	DKFZp434K0621	Homo sapiens mRNA; cDNA DKFZp434K0621 (from clone DKFZp434K0621); partial cds
268	AI168147		Homo sapiens HSPC289 mRNA, partial cds

269	L02326		Homo sapiens clone Hu lambda7 lambda-like protein (IGLL2) gene, partial cds
270	F09520	EST	Homo sapiens clone 24841 mRNA sequence
271	AA975205		Homo sapiens clone 23570 mRNA sequence
272	AI348289		Homo sapiens cDNA: FLJ23227 fis, clone CAE00645, highly similar to AF052138 Homo sapiens clone 23718 mRNA sequence
273	AA669034		Homo sapiens cDNA: FLJ23125 fis, clone LNG08217
274	W76303		Homo sapiens cDNA: FLJ22662 fis, clone HSI08080
275	T04932		Homo sapiens cDNA: FLJ21545 fis, clone COL06195
276	AA147751		Homo sapiens cDNA FLJ14146 fis, clone MAMMA1002947
277	N91027		Homo sapiens cDNA FLJ13549 fis, clone PLACE1007097
278	AA188494	FLJ113352	Homo sapiens cDNA FLJ13352 fis, clone OVARC1002165, weakly similar to 3-OXO-5-ALPHA-STEROID 4-DEHYDROGENASE 2 (EC 1.3.99.5)
279	AA903456		Homo sapiens cDNA FLJ13325 fis, clone OVARC1001762, weakly similar to N-TERMINAL ACETYLTRANSFERASE 1 (EC 2.3.1.88)
280	AA628522		Homo sapiens cDNA FLJ12758 fis, clone NT2RP2001328
281	AA626414		Homo sapiens cDNA FLJ12436 fis, clone NT2RM1000062
282	AA610175	FLJ12195	Homo sapiens cDNA FLJ12195 fis, clone MAMMA1000865
283	AW083127		Homo sapiens cDNA FLJ11856 fis, clone HEMBA1006789
284	F18016		Homo sapiens cDNA FLJ11018 fis, clone PLACE1003602, highly similar to Homo sapiens mRNA expressed in plcenta
285	AA442071	EST	Homo sapiens cDNA FLJ10247 fis, clone HEMBB1000705
286	AA036947		Homo sapiens cDNA FLJ10229 fis, clone HEMBB1000136
287	AA234475	NCOA6IP	PRIP-interacting protein with methyltransferase domain

288	AI041186		HSPC182 protein
289	K01505		DC classII histocompatibility antigen alpha-chain
290	Z38677		Claudin 10
291	AA236315		Chromosome 1 open reading frame 27
292	AA411333		ESTs, Weakly similar to zinc finger-like [H.sapiens]
293	AA150200		ESTs, Weakly similar to tuftelin [M.musculus]
294	AI341906		ESTs, Weakly similar to ORF YNL310c [S.cerevisiae]
295	AI349804	EST	ESTs, Weakly similar to IQGA_HUMAN RAS GTPASE-ACTIVATING-LIKE PROTEIN IQGAP1 [H.sapiens]
296	W94363		ESTs, Weakly similar to ALU4_HUMAN ALU SUBFAMILY SB2 SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]
297	AA053248		ESTs, Highly similar to RS10_HUMAN 40S RIBOSOMAL PROTEIN S10 [H.sapiens]
298	AA514648		ESTs, Highly similar to LMA1_HUMAN LAMININ ALPHA CHAIN PRECURSOR [H.sapiens]
299	T03298		ESTs, Highly similar to LDHH_HUMAN L-LACTATE DEHYDROGENASE H CHAIN [H.sapiens]
300	T55019		ESTs, fetal spleen
301	AI088718		ESTs
302	AA024920		ESTs
303	R77448	PLXNA2	ESTs
304	W31174		ESTs
305	AA463626		ESTs
306	AI344249		ESTs
307	R61891		ESTs
308	AA479350		ESTs
309	AA327207		ESTs
310	AA528140		ESTs
311	AA826148	EST	ESTs
312	AA913950		ESTs
313	AI243620		ESTs
314	AI039201		ESTs
315	AA936889		ESTs
316	AA687757		ESTs

317	AI366259		ESTs
318	AA317670		ESTs
319	AI141923		ESTs
320	AA778238	EST	ESTs
321	T72555		ESTs
322	AA602585		ESTs
323	AA527570		ESTs
324	C75253		ESTs
325	AA351680		ESTs
326	N75945		ESTs
327	AA528243		ESTs
328	AA688195		ESTs
329	AA063157		ESTs
330	AA419568		ESTs
331	D85376		ESTs
332	AA521342		ESTs
333	AI365844		ESTs
334	T55926		ESTs
335	R94687		ESTs
336	T61564		ESTs
337	AI305234	LOC152217	ESTs
338	AA233870		ESTs
339	T16470		ESTs
340	T16802		ESTs
341	AA830668	EST	EST
342	AA489212		EST
343	AA758394		EST
344	AA609658		EST
345	AA683373		EST
346	N34387		EST

Table 4 593 genes commomly 0.2 fold down-regulated or less in testicular seminomas.

TS Assignment	Accession No.	Symbol	Gene name
347	U57961	13CDNA73	putative gene product
348	M35296	ABL2	v-abl Abelson murine leukemia viral oncogene homolog 2 (arg, Abelson-related gene)
349	AA406601	ABLIM	actin binding LIM protein 1
350	AA815365	ACT	activator of CREM in testis
351	AI357650	AD026	AD026 protein
352	AF029900	ADAM21	a disintegrin and metalloproteinase domain 21
353	X74210	ADCY2	adenylate cyclase 2 (brain)
354	X03350	ADH2	alcohol dehydrogenase 2 (class I), beta polypeptide
355	L22214	ADORA1	adenosine A1 receptor
356	X66503	ADSS	adenylosuccinate synthase

357	AA766028	AF15Q14	AF15q14 protein
358	AA434178	AGPAT1	1-acylglycerol-3-phosphate O-acyltransferase 1 (lysophosphatidic acid acyltransferase, alpha)
359	AF038564	AIP4	atrophin interacting protein 4
360	AI028271	AKAP3	A kinase (PRKA) anchor protein 3
361	AA398240	AKAP4	A kinase (PRKA) anchor protein 4
362	U05861	AKR1C1	aldo-keto reductase family 1, member C1 (dihydrodiol dehydrogenase 1; 20-alpha (3-alpha)-hydroxysteroid dehydrogenase)
363	D17793	AKR1C3	aldo-keto reductase family 1, member C3 (3-alpha hydroxysteroid dehydrogenase, type II)
364	K03000	ALDH1	aldehyde dehydrogenase 1, soluble
365	M18786	AMY1A	amylase, alpha 1A; salivary
366	M19383	ANXA4	annexin A4
367	Y12226	AP1G1	adaptor-related protein complex 1, gamma 1 subunit
368	AI278652	AP1S2	adaptor-related protein complex 1, sigma 2 subunit
369	AA421206	APG	heat shock protein (hsp110 family)
370	AI168526	ARHGAP5	Rho GTPase activating protein 5
371	AI025137	ARHGEF3	Rho guanine nucleotide exchange factor (GEF) 3
372	AB002305	ARNT2	aryl-hydrocarbon receptor nuclear translocator 2
373	U47054	ART3	ADP-ribosyltransferase 3
374	AA928117	ATP8A2	ATPase, aminophospholipid transporter-like, Class I, type 8A, member 2
375	H80325	BAZ1A	bromodomain adjacent to zinc finger domain, 1A
376	M55575	BCKDHB	branched chain keto acid dehydrogenase E1, beta polypeptide (maple syrup urine disease)
377	D87461	BCL2L2	BCL2-like 2
378	AA620708	BCLG	Apoptosis regulator BCL-G
379	U70824	BLu	BLu protein
380	AA916688	BRF1	butyrate response factor 1 (EGF-response factor 1)

381	U03274	BTD	biotinidase
382	D31716	BTEB1	basic transcription element binding protein 1
383	W45244	C3	complement component 3
384	U36448	CADPS	Ca2+-dependent activator protein for secretion
385	X56667	CALB2	calbindin 2, (29kD, calretinin)
386	AA600048	CALD1	caldesmon 1
387	R39610	CAPN2	calpain 2, (m/II) large subunit
388	AI085802	CAV2	Caveolin 2
389	M58583	CBLN1	cerebellin 1 precursor
390	D78333	CCT6B	chaperonin containing TCP1, subunit 6B (zeta 2)
391	AA917718	CDC10	CDC10 (cell division cycle 10, S. cerevisiae, homolog)
392	L27711	CDKN3	cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)
393	AI140736	CDV	CDV protein
394	AF083322	CEP1	centrosomal protein 1
395	AI142230	CETN3	centrin, EF-hand protein, 3 (CDC31 yeast homolog)
396	J03483	CHGA	chromogranin A (parathyroid secretory protein 1)
397	D10704	CHK	choline kinase
398	AA400791	CHST3	Carbohydrate (chondroitin 6/keratan) sulfotransferase 3
399	U65092	CITED1	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1
400	AI333035	CKAP2	cytoskeleton associated protein 2
401	AI078139	CKN1	Cockayne syndrome 1 (classical)
402	D86322	CLGN	calmegin
403	M64722	CLU	clusterin (complement lysis inhi bitor, SP-40,40, sulfated glycop rotein 2, testosterone-repressed prostate message 2, apolipoprot ein J)
404	D17408	CNN1	calponin 1, basic, smooth muscle
405	L25286	COL15A1	collagen, type XV, alpha 1
406	T93566	CPE	carboxypeptidase E
407	F21182	CRAT	carnitine acetyltransferase
408	AI334396	CRSP9	cofactor required for Sp1 transcriptional activation, subunit 9 (33kD)
409	M55268	CSNK2A2	casein kinase 2, alpha prime polypeptide
410	X16312	CSNK2B	casein kinase 2, beta polypeptide
411	U16306	CSPG2	chondroitin sulfate proteoglycan 2

			(versican)
412	M33146	CSRP1	cysteine and glycine-rich protein 1
413	AA780301	CTSF	cathepsin F
414	AB001928	CTSL2	cathepsin L2
415	AA417733	CUL1	cullin 1
416	Z22780	CYLC1	cylicin, basic protein of sperm head cytoskeleton 1
417	M14564	CYP17	cytochrome P450, subfamily XVII (steroid 17-alpha-hydroxylase), adrenal hyperplasia
418	U62015	CYR61	cysteine-rich, angiogenic inducer, 61
419	AA608804	D6S51E	HLA-B associated transcript-2
420	AA640753	DDAH1	dimethylarginine dimethylaminohydrolase 1
421	X62535	DGKA	diacylglycerol kinase, alpha (80kD)
422	AI209130	DJ402G11.8	novel protein similar to mouse MOV10
423	AA432207	DMRT1	doublesex and mab-3 related transcription factor 1
424	AJ000522	DNAH17	dynein, axonemal, heavy polypeptide 17
425	U53445	DOC1	downregulated in ovarian cancer 1
426	AA488466	DRG1	developmentally regulated GTP-binding protein 1
427	X68277	DUSP1	dual specificity phosphatase 1
428	AA313118	DUSP10	dual specificity phosphatase 10
429	U89278	EDR2	early development regulator 2 (homolog of polyhomeotic 2)
430	M62829	EGR1	early growth response 1
431	AA398573	EIF5A2	eukaryotic translation initiation factor 5A2
432	AI097529	EPAS1	endothelial PAS domain protein 1
433	U62740	EXT2	exostoses (multiple) 2
434	M14354	F13A1	coagulation factor XIII, A1 polypeptide
435	D10040	FACL2	fatty-acid-Coenzyme A ligase, long-chain 2
436	L13923	FBN1	fibrillin 1 (Marfan syndrome)
437	AI194045	FE65L2	FE65-LIKE 2
438	AI351061	FEM1B	FEM (C.elegans) homolog b
439	D14446	FGL1	fibrinogen-like 1
440	U60115	FHL1	four and a half LIM domains 1
441	AA678103	FKBP5	FK506-binding protein 5
442	L37033	FKBP8	FK506-binding protein 8 (38kD)
443	AA876478	FLJ10578	Sec61 alpha form 2

444	AI141417	FLJ10873	UDP-glucose:glycoprotein glucosyltransferase 2
445	AA813008	FOP	FGFR1 oncogene partner
446	X74142	FOXG1B	forkhead box G1B
447	AI025916	FSP-2	fibrousheathin II
448	X03674	G6PD	glucose-6-phosphate dehydrogenase
449	N34138	GABARAP	GABA(A) receptor-associated protein
450	U13044	GABPA	GA-binding protein transcription factor, alpha subunit (60kD)
451	S68805	GATM	glycine amidinotransferase (L-arginine:glycine amidinotransferase)
452	AA583339	GCNT3	glucosaminyl (N-acetyl) transferase 3, mucin type
453	AI014575	GCP60	golgi resident protein GCP60
454	AA578014	GGA1	ADP-ribosylation factor binding protein GGA1
455	AA523541	GILZ	glucocorticoid-induced leucine zipper
456	AA293636	GJA1	gap junction protein, alpha 1, 43kD (connexin 43)
457	AA608780	GKP2	Glycerol kinase pseudogene 2
458	AA887118	GLRX2	Glutaredoxin 2
459	AA446421	GMPS	guanine monphosphate synthetase
460	AF055013	GNAI1	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1
461	AA401492	GNAS1	guanine nucleotide binding protein (G protein), alpha stimulating activity polypeptide 1
462	AF007548	GOSR2	golgi SNAP receptor complex member 2
463	AA031372	GPC4	glypican 4
464	AI126171	GPP130	type II Golgi membrane protein
465	L42324	GPR18	G protein-coupled receptor 18
466	X71973	GPX4	glutathione peroxidase 4 (phospholipid hydroperoxidase)
467	L76687	GRB14	growth factor receptor-bound protein 14
468	AI015487	GRTH	gonadotropin-regulated testicular RNA helicase
469	D87119	GS3955	GS3955 protein
470	AA993251	GSTA2	glutathione S-transferase A2
471	L13275	GSTA3	glutathione S-transferase A3
472	L02321	GSTM5	glutathione S-transferase M5
473	U14193	GTF2A2	general transcription factor IIA, 2 (12kD subunit)

474	AI126491	HBACH	Cytosolic acyl coenzyme A thioester hydrolase
475	AF019214	HBP1	HMG-box containing protein 1
476	W95267	HIBADH	3-hydroxyisobutyrate dehydrogenase
477	U40992	HLJ1	DnaJ-like heat shock protein 40
478	M11058	HMGCR	3-hydroxy-3-methylglutaryl-Coenzyme A reductase
479	X83618	HMGCS2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)
480	AI215478	HMMR	hyaluronan-mediated motility receptor (RHAMM)
481	Y09980	HOXD3	homeo box D3
482	AF070616	HPCAL1	hippocalcin-like 1
483	Y12711	HPR6.6	progesterone binding protein
484	AA825654	HRB	HIV Rev binding protein
485	AI027700	HS1-2	putative transmembrane protein
486	M65217	HSF2	heat shock transcription factor 2
487	AI205684	HSPA2	heat shock 70kD protein 2
488	AA971601	HSSOX6	SRY (sex determining region Y)-box 6
489	AA493561	IGSF4	immunoglobulin superfamily, member 4
490	AA916823	IL1A	interleukin 1, alpha
491	M27492	IL1R1	interleukin 1 receptor, type I
492	D61009	ING1L	inhibitor of growth family, member 1-like
493	L08488	INPP1	inositol polyphosphate-phosphatase
494	AI192189	INPP5A	inositol polyphosphate-5-phosphatase, 40kD
495	W76477	JUN	v-jun avian sarcoma virus 17 oncogene homolog
496	AA933702	KCNK4	potassium inwardly-rectifying channel, subfamily K, member 4
497	U25138	KCNMB1	potassium large conductance calcium-activated channel, subfamily M, beta member 1
498	AF064093	KEO4	similar to Caenorhabditis elegans protein C42C1.9
499	D14661	KIAA0105	Wilms' tumour 1-associating protein
500	AB014531	KIAA0631	very long-chain acyl-CoA synthetase; lipidosin
501	H98203	KIAA0987	differentially expressed in adenocarcinoma of the lung
502	AA037452	KIAA0992	palladin
503	Y08319	KIF2	kinesin heavy chain member 2

504	AL044356	KPNB3	karyopherin (importin) beta 3
505	M59832	LAMA2	laminin, alpha 2 (merosin, congenital muscular dystrophy)
506	AF064492	LDB2	LIM domain binding 2
507	L13210	LGALS3BP	lectin, galactoside-binding, soluble, 3 binding protein (galectin 6 binding protein)
508	AA252389	LHFP	lipoma HMGIC fusion partner
509	AA191662	LOC51617	HMP19 protein
510	AI160184	LOC51673	brain specific protein
511	AA569922	LOC51706	cytochrome b5 reductase 1 (B5R.1)
512	AA527435	LOC63928	hepatocellular carcinoma antigen gene 520
513	AA173168	LRRFIP2	leucine rich repeat (in FLII) interacting protein 2
514	M83202	LTF	lactotransferrin
515	AA459595	LZK1	C3HC4-type zinc finger protein
516	U44378	MADH4	MAD (mothers against decapentaplegic, Drosophila) homolog 4
517	X74837	MAN1A1	mannosidase, alpha, class 1A, member 1
518	M69226	MAOA	monoamine oxidase A
519	AA157731	MAP1ALC3	Microtubule-associated proteins 1A and 1B, light chain 3
520	U07620	MAPK10	mitogen-activated protein kinase 10
521	D10511	MAT	mitochondrial acetoacetyl-CoA thiolase
522	X68836	MAT2A	methionine adenosyltransferase II, alpha
523	AA228022	MCAM	melanoma adhesion molecule
524	X12556	MCF2	MCF.2 cell line derived transforming sequence
525	AI215620	MCSP	mitochondrial capsule selenoprotein
526	AA815051	MDG1	microvascular endothelial differentiation gene 1
527	L38486	MFAP4	microfibrillar-associated protein 4
528	AA135566	MGEA6	meningioma expressed antigen 6 (coiled-coil proline-rich)
529	X53331	MGP	matrix Gla protein
530	U77604	MGST2	microsomal glutathione S-transferase 2
531	M16279	MIC2	antigen identified by monoclonal antibodies 12E7, F21 and O13
532	U38320	MMP19	matrix metalloproteinase 19

533	M93405	MMSDH	methylmalonate-semialdehyde dehydrogenase
534	AI140756	MP1	metalloprotease 1 (pitrilysin family)
535	AA868815	MSL3L1	male-specific lethal-3 (Drosophila)-like 1
536	X59657	MTP	microsomal triglyceride transfer protein (large polypeptide, 88kD)
537	J05581	MUC1	mucin 1, transmembrane
538	AA401638	MUL	Mulibrey nanism
539	AA319638	MYH9	Myosin, heavy polypeptide 9, non-muscle
540	X85337	MYLK	myosin, light polypeptide kinase
541	D87930	MYPT1	myosin phosphatase, target subunit 1
542	J02854	MYRL2	myosin regulatory light chain 2, smooth muscle isoform
543	D50370	NAP1L3	nucleosome assembly protein 1-like 3
544	AA906200	NAP4	Nck, Ash and phospholipase C binding protein
545	AA855085	NCOA4	nuclear receptor coactivator 4
546	U22897	NDP52	nuclear domain 10 protein
547	AI088622	NDUFS2	NADH dehydrogenase (ubiquinone) Fe-S protein 2 (49kD) (NADH-coenzyme Q reductase)
548	Y00067	NEF3	neurofilament 3 (150kD medium)
549	M58603	NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)
550	U83843	NIP7-1	HIV-1 Nef interacting protein
551	AA707108	NKX3A	NK homeobox (Drosophila), family 3, A
552	AA340728	NR2F2	nuclear receptor subfamily 2, group F, member 2
553	AA215284	NSF	N-ethylmaleimide-sensitive factor
554	X55740	NT5	5' nucleotidase (CD73)
555	X76732	NUCB2	nucleobindin 2
556	AJ007558	NUP155	nucleoporin 155kD
557	AA902823	NYD-SP12	NYD-SP12 protein
558	AA699559	NYD-SP15	Protein kinase NYD-SP15
559	AI208877	NYD-SP21	Testes development-related NYD-SP21
560	AA729034	ODC1	ornithine decarboxylase 1
561	AF012549	ODF2	outer dense fibre of sperm tails 2
562	AA889218	OGN	osteoglycin (osteoinductive factor, mimecan)

563	AA922747	OXR1	oxidation resistance 1
564	M37721	PAM	peptidylglycine alpha-amidating monooxygenase
565	X76770	PAP	poly(A) polymerase
566	U02020	PBEF	pre-B-cell colony-enhancing factor
567	AA626775	PCDHA5	protocadherin alpha 5
568	D84307	PCYT2	phosphate cytidylyltransferase 2, ethanolamine
569	AA004890	PDCD8	programmed cell death 8 (apoptosis-inducing factor)
570	AA400893	PDE1A	phosphodiesterase 1A, calmodulin-dependent
571	AI192411	PDGFRA	platelet-derived growth factor receptor, alpha polypeptide
572	C05229	PDK4	pyruvate dehydrogenase kinase, isoenzyme 4
573	U79296	PDX1	Pyruvate dehydrogenase complex, lipoyl-containing component X; E3-binding protein
574	J00123	PENK	proenkephalin
575	AF048755	PEX13	peroxisome biogenesis factor 13
576	D25328	PFKP	phosphofructokinase, platelet
577	W58700	PHKB	phosphorylase kinase, beta
578	AA057243	PHRET1	PH domain containing protein in retina 1
579	AA515710	PIGN	phosphatidylinositol glycan, class N
580	AA634825	PINK1	PTEN induced putative kinase 1
581	U09117	PLCD1	phospholipase C, delta 1
582	AA777648	PMP22	peripheral myelin protein 22
583	AF023455	PPEF1	protein phosphatase, EF hand calcium-binding domain 1
584	AF034803	PPFIBP2	PTPRF interacting protein, binding protein 2 (liprin beta 2)
585	Z50749	PPP1R7	protein phosphatase 1, regulatory subunit 7
586	M60484	PPP2CB	protein phosphatase 2 (formerly 2A), catalytic subunit, beta isoform
587	U37352	PPP2R5C	protein phosphatase 2, regulatory subunit B (B56), gamma isoform
588	AI299911	PPP3CA	protein phosphatase 3 (formerly 2B), catalytic subunit, alpha isoform (calcineurin A alpha)
589	N29328	PPP4R1	protein phosphatase 4, regulatory subunit 1
590	X75756	PRKCM	protein kinase C, mu
591	AI357236	PRM1	protamine 1

592	X07862	PRM2	protamine 2
593	AI242370	PRND	prion gene complex, downstream
594	U51990	PRP18	pre-mRNA splicing factor similar to S. cerevisiae Prp18
595	Y00971	PRPS2	phosphoribosyl pyrophosphate synthetase 2
596	D87258	PRSS11	protease, serine, 11 (IGF binding)
597	M61900	PTGDS	prostaglandin D synthase gene
598	M57399	PTN	pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)
599	W84417	RANBP9	RAN binding protein 9
600	AA635922	RANGAP1	Ran GTPase activating protein 1
601	AB008109	RGS5	regulator of G-protein signalling 5
602	AA778308	RNASE1	ribonuclease, RNase A family, 1 (pancreatic)
603	AA854469	RNF6	ring finger protein (C3H2C3 type) 6
604	AI095724	RPL17	ribosomal protein L17
605	AF056929	SARCOSIN	sarcomeric muscle protein
606	Y13647	SCD	stearoyl-CoA desaturase (delta-9-desaturase)
607	AJ224677	SCRG1	scrapie responsive protein 1
608	T36260	SEC23B	Sec23 (S. cerevisiae) homolog B
609	AA401227	SEC31B-1	Secretory pathway component Sec31B-1
610	AA703667	SEC8	secretory protein, SEC8
611	AI026695	SENP1	Sentrin/SUMO-specific protease
612	Z11793	SEPP1	selenoprotein P, plasma, 1
613	AF042081	SH3BGRL	SH3 domain binding glutamic acid-rich protein like
614	AF036269	SH3GL3	SH3-domain GRB2-like 3
615	T35854	SIAH2	seven in absentia (Drosophila) homolog 2
616	N53491	SIRT3	sir2-like 3
617	AA639599	SLC12A2	solute carrier family 12 (sodium/potassium/chloride transporters), member 2
618	N30856	SLC19A2	solute carrier family 19 (thiamine transporter), member 2
619	M55531	SLC2A5	solute carrier family 2 (facilitated glucose transporter), member 5
620	AA838741	SLC35A1	Solute carrier family 35 (CMP-sialic acid transporter), member 1
621	AA758636	SMAP	Thyroid hormone receptor coactivating protein
622	M88163	SMARCA1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily

			a, member 1
623	W70141	SMARCA3	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 3
624	AI222903	SMARCD2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 2
625	AI351686	SMOC1	secreted modular calcium-binding protein 1
626	AA946930	SNRPG	small nuclear ribonucleoprotein polypeptide G
627	W56480	SOS1	son of sevenless (Drosophila) homolog 1
628	Z46629	SOX9	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)
629	AA760720	SPAG6	sperm associated antigen 6
630	AI459767	SPARCL1	SPARC-like 1 (mast9, hevin)
631	AA779272	SPINK2	serine protease inhibitor, Kazal type, 2 (acrosin-trypsin inhibitor)
632	M61199	SSFA2	sperm specific antigen 2
633	AI024234	SSTK	Serine/threonine protein kinase SSTK
634	U17280	STAR	steroidogenic acute regulatory protein
635	U14550	STHM	sialyltransferase
636	L77564	STK22B	serine/threonine kinase 22B (spermiogenesis associated)
637	AA935437	STRIN	STRIN protein
638	H10341	SULTX3	sulfotransferase-related protein
639	AA643682	SUV39H2	Suppressor of variegation 3-9 (Drosophila) homolog 2; hypothetical protein FLJ23414
640	Z21437	TAF2G	TATA box binding protein (TBP)-associated factor, RNA polymerase II, G, 32kD
641	AI093734	TAZ	Transcriptional co-activator with PDZ-binding motif (TAZ)
642	AA628669	TBL2	transducin (beta)-like 2
643	AI243203	TEX14	Testis expressed sequence 14
644	S95936	TF	transferrin
645	AA573143	TIMP2	tissue inhibitor of metalloproteinase 2
646	AI086204	TM4SF6	transmembrane 4 superfamily member 6
647	U81006	TM9SF2	transmembrane 9 superfamily member 2

648	L01042	TMF1	TATA element modulatory factor 1
649	X64559	TNA	tetranectin (plasminogen-binding protein)
650	X07948	TNP1	transition protein 1 (during histone to protamine replacement)
651	J04088	TOP2A	topoisomerase (DNA) II alpha (170kD)
652	U54831	TOP2B	topoisomerase (DNA) II beta (180kD)
653	AA913471	TOPK	PDZ-binding kinase; T-cell originated protein kinase
654	X66397	TPR	translocated promoter region (to activated MET oncogene)
655	M25532	TPX1	testis specific protein 1 (probe H4 p3)
656	X63679	TRAM	translocating chain-associating membrane protein
657	AF064801	TRC8	patched related protein translocated in renal cancer
658	AI346969	TRIM14	Tripartite motif-containing 14
659	AF065388	TSPAN	tetraspan 1
660	AA432312	TSPYL	TSPY-like
661	AA456299	T-STAR	Sam68-like phosphotyrosine protein, T-STAR
662	X69490	TTN	titin
663	AA709190	TUBA2	tubulin, alpha 2
664	X02308	TYMS	thymidylate synthetase
665	AI344684	UBE2N	ubiquitin-conjugating enzyme E2N (homologous to yeast UBC13)
666	AA416852	UBL3	ubiquitin-like 3
667	N44888	UPF3A	similar to yeast Upf3, variant A
668	AA116022	USP18	ubiquitin specific protease 18
669	AA846445	USP6	ubiquitin specific protease 6 (Tre-2 oncogene)
670	BG028760	USP7	ubiquitin specific protease 7 (herpes virus-associated)
671	T29210	UTRN	utrophin (homologous to dystrophin)
672	AI018129	VAMP4	vesicle-associated membrane protein 4
673	D87459	WASF1	WAS protein family, member 1
674	S69790	WASF3	WAS protein family, member 3
675	AA364135	WDR10	WD repeat domain 10
676	AA160764	WHSC1	Wolf-Hirschhorn syndrome candidate 1
677	X51630	WT1	Wilms tumor 1
678	W55933	WW45	WW Domain-Containing Gene

679	N66453	XPC	xeroderma pigmentosum, complementation group C
680	D83407	ZAKI4	Down syndrome critical region gene 1-like 1
681	M92843	ZFP36	zinc finger protein homologous to Zfp-36 in mouse
682	X84801	ZNF165	zinc finger protein 165
683	AF017433	ZNF213	zinc finger protein 213
684	AA703988	ZNF259	zinc finger protein 259
685	AA897714	ZNF6	Zinc finger protein 6 (CMPX1)
686	U54996	ZW10	ZW10 (Drosophila) homolog, centromere/kinetochore protein
687	AA936961	LOC57032	similar to acetyl-coenzyme A synthetase
688	AA234377	CL25022	hypothetical protein
689	N35437	DJ1181N3.1	hypothetical protein dJ1181N3.1
690	Z20328	DKFZp434C 0328	hypothetical protein DKFZp434C0328
691	H19830	DKFZP434G 156	hypothetical protein DKFZp434G156
692	AI127752	DKFZP434I0 92	DKFZP434I092 protein
693	T65389	DKFZP434J2 14	DKFZP434J214 protein
694	AA284134	DKFZP434L 243	DKFZP434L243 protein
695	AI192351	DKFZP564B 167	DKFZP564B167 protein
696	AA865478	DKFZP564J0 863	DKFZP564J0863 protein
697	AI306435	DKFZP586A 0522	DKFZP586A0522 protein
698	AA709155	FLJ10134	hypothetical protein FLJ10134
699	AA582581	FLJ10159	hypothetical protein FLJ10159
700	AI076154	FLJ10283	hypothetical protein FLJ10283
701	AA759066	FLJ10392	hypothetical protein FLJ10392
702	AA452368	FLJ10582	hypothetical protein FLJ10582
703	U69201	FLJ10761	hypothetical protein FLJ10761
704	AA418149	FLJ10850	hypothetical protein FLJ10850
705	AA775271	FLJ10914	hypothetical protein FLJ10914
706	AA293776	FLJ10921	hypothetical protein FLJ10921
707	AI221110	FLJ10980	hypothetical protein FLJ10980
708	AA634293	FLJ11088	hypothetical protein FLJ11088
709	D81610	FLJ11109	hypothetical protein FLJ11109
710	AA056538	FLJ11210	hypothetical protein FLJ11210
711	AA781142	FLJ11307	hypothetical protein FLJ11307
712	AA214211	FLJ13110	hypothetical protein FLJ13110
713	AI147953	FLJ20010	hypothetical protein
714	C00491	FLJ20121	hypothetical protein FLJ20121

715	AK024920	FLJ20152	hypothetical protein
716	AA634416	FLJ20425	hypothetical protein FLJ20425
717	AA809070	FLJ20535	hypothetical protein FLJ20535
718	H20535	FLJ21324	hypothetical protein FLJ21324
719	AI346388	FLJ21347	hypothetical protein FLJ21347
720	AI016734	FLJ22104	hypothetical protein FLJ22104
721	AA677445	H41	hypothetical protein
722	AA126461	HSA272196	hypothetical protein, clone 2746033
723	AI003803	HSD-3.1	hypothetical protein
724	AI300283	IMPACT	hypothetical protein IMPACT
725	D38521	KIAA0077	KIAA0077 protein
726	D86984	KIAA0231	KIAA0231 protein
727	D87438	KIAA0251	KIAA0251 protein
728	D87465	KIAA0275	KIAA0275 gene product
729	AF007170	KIAA0452	DEME-6 protein
730	AA910738	KIAA0579	KIAA0579 protein
731	N30392	KIAA0608	KIAA0608 protein
732	AB014534	KIAA0634	KIAA0634 protein
733	AI167680	KIAA0643	Homo sapiens cDNA FLJ13257 fis, clone OVARC1000846, weakly similar to NUCLEOLIN
734	AA506972	KIAA0668	KIAA0668 protein
735	AA665890	KIAA0729	KIAA0729 protein
736	N49366	KIAA0737	KIAA0737 gene product
737	H09503	KIAA0740	KIAA0740 gene product
738	AF052170	KIAA0750	KIAA0750 gene product
739	AA234129	KIAA0863	KIAA0863 protein
740	AA399583	KIAA0874	KIAA0874 protein
741	H03641	KIAA0914	KIAA0914 gene product
742	AI253232	KIAA0996	KIAA0996 protein
743	AA339816	KIAA1028	KIAA1028 protein
744	AI187395	KIAA1053	KIAA1053 protein
745	AA056734	KIAA1110	KIAA1110 protein
746	AI217997	KIAA1128	KIAA1128 protein
747	AA037467	KIAA1165	hypothetical protein KIAA1165
748	AA994997	KIAA1223	KIAA1223 protein
749	W68261	KIAA1327	KIAA1327 protein
750	AA781940	KIAA1336	KIAA1336 protein
751	AI082425	KIAA1430	KIAA1430 protein
752	AI243817	KIAA1494	Homo sapiens cDNA: FLJ23073 fis, clone LNG05726
753	AA824313	KIAA1505	KIAA1505 protein
754	D59339	KIAA1529	Homo sapiens mRNA; cDNA DKFZp434I2420 (from clone DKFZp434I2420)
755	AA044905	KIAA1596	KIAA1596 protein
756	T34177	LOC51255	hypothetical protein
757	AA776749	LOC57821	hypothetical protein LOC57821

758	R00068	PRO1580	hypothetical protein PRO1580
759	AI302506	PRO1912	PRO1912 protein
760	AF113020	PRO2463	PRO2463 protein
761	AI218544	FLJ20425	hypothetical protein FLJ20425
762	AI214973	KIAA1223	KIAA1223 protein
763	AI215074		Homo sapiens cDNA FLJ11095 fis, clone PLACE1005374
764	AA587860		Homo sapiens cDNA FLJ11205 fis, clone PLACE1007843
765	AA043562		Homo sapiens cDNA FLJ11667 fis, clone HEMBA1004697
766	AI277493		Homo sapiens cDNA FLJ11756 fis, clone HEMBA1005595, weakly similar to DYNEIN HEAVY CHAIN, CYTOSOLIC
767	AI078809		Homo sapiens cDNA FLJ12627 fis, clone NT2RM4001813, weakly similar to LECTIN BRA-2
768	AI028392		Homo sapiens cDNA FLJ13229 fis, clone OVARC1000106
769	AA830551		Homo sapiens cDNA FLJ13848 fis, clone THYRO1000855
770	AA853955		Homo sapiens cDNA FLJ13992 fis, clone Y79AA1002139, weakly similar to DNAJ PROTEIN HOMOLOG 1
771	AA320463		Homo sapiens cDNA: FLJ21127 fis, clone CAS06212
772	AA393838		Homo sapiens cDNA: FLJ21849 fis, clone HEP01928
773	AA400674		Homo sapiens cDNA: FLJ21962 fis, clone HEP05564
774	AA148493		Homo sapiens cDNA: FLJ22300 fis, clone HRC04759
775	AA411157		Homo sapiens cDNA: FLJ22448 fis, clone HRC09541
776	AA631197		Homo sapiens cDNA: FLJ22477 fis, clone HRC10815
777	T65582		Homo sapiens cDNA: FLJ22637 fis, clone HSI06677
778	AI192127		Homo sapiens cDNA: FLJ22712 fis, clone HSI13435
779	AA148566		Homo sapiens cDNA: FLJ22790 fis, clone KAIA2176, highly similar to HUMPMCA Human plasma membrane calcium-pumping ATPase (PMCA4) mRNA

780	AA633352	Homo sapiens cDNA: FLJ23067 fis, clone LNG04993
781	AI084531	Homo sapiens cDNA: FLJ23093 fis, clone LNG07264
782	AA450190	Homo sapiens cDNA: FLJ23316 fis, clone HEP12031
783	AA975521	Homo sapiens cDNA: FLJ23518 fis, clone LNG04878
784	AI097058	Homo sapiens cDNA: FLJ23538 fis, clone LNG08010, highly similar to BETA2 Human MEN1 region clone epsilon/beta mRNA
785	AA405953	Homo sapiens chromosome 11 unknown mRNA sequence
786	N32181	Homo sapiens clone 25056 mRNA sequence
787	AA262802	Homo sapiens clone SP329 unknown mRNA
788	AA293837	Homo sapiens GKAP42 (FKSG21) mRNA, complete cds
789	AA970955	Homo sapiens mRNA; cDNA DKFZp434B0610 (from clone DKFZp434B0610); partial cds
790	AA843455	Homo sapiens mRNA; cDNA DKFZp434E232 (from clone DKFZp434E232)
791	AA421199	Homo sapiens mRNA; cDNA DKFZp434L0217 (from clone DKFZp434L0217); partial cds
792	AA393597	Homo sapiens mRNA; cDNA DKFZp434P2072 (from clone DKFZp434P2072); partial cds
793	AA976808	Homo sapiens mRNA; cDNA DKFZp564C046 (from clone DKFZp564C046)
794	AI280901	Homo sapiens mRNA; cDNA DKFZp564D016 (from clone DKFZp564D016)
795	AA443685	Homo sapiens mRNA; cDNA DKFZp564H142 (from clone DKFZp564H142)
796	N41310	Homo sapiens mRNA; cDNA DKFZp564P046 (from clone DKFZp564P046)
797	AI299718	Homo sapiens mRNA; cDNA DKFZp586B1922 (from clone DKFZp586B1922)
798	AA280818	Homo sapiens mRNA; cDNA DKFZp586G2222 (from clone

			DKFZp586G2222)
799	AI150152		Homo sapiens PAC clone RP5-981O7 from 7q34-q36
800	AI016755		Homo sapiens ropporin mRNA, complete cds
801	AI014769		Homo sapiens TRAF4 associated factor 1 mRNA, partial cds
802	AA004698		Homo sapiens ubiquitin-like fusion protein mRNA, complete cds
803	AA431698		Human DNA sequence from clone 1068E13 on chromosome 20p11.212.3. Contains two putative novel genes, the gene for a novel protein similar to bovine SCP2 (Sterol Carrier Protein 2) and part of HSD17B4 (hydroxysteroid (17-beta) dehydrogenase 4), an EEF1A1 (
804	AA126472		Human DNA sequence from clone 747H23 on chromosome 6q135. Contains the 3' part of the ME1 gene for malic enzyme 1, soluble (NADP-dependent malic enzyme, malate oxidoreductase, EC 1.1.1.40), a novel gene and the 5' part of the gene for N-acetylglucosamine
805	AA651872		Human DNA sequence from clone RP12G14 on chromosome 6q24.1-25.2. Contains the 5' end of the gene for a novel cyclophilin type peptidyl-prolyl cis-trans isomerase, a novel gene, an RPS18 (40S Ribosomal protein S18) pseudogene, the 3' end of the KATNA1 gen
806	A25270		IFN-gamma antagonist cytokine
807	AA650281		Likely ortholog of mouse tumor necrosis-alpha-induced adipose-related protein
808	AI015633		Solute carrier family 26, member 8
809	N47682	KIAA1673	ESTs
810	AA578684	KIAA1674	ESTs
811	Z21254	KIAA1771	ESTs, Weakly similar to unnamed protein product [H.sapiens]
812	R61253	KIAA1877	ESTs

813	W67209	KIAA0251	ESTs, Moderately similar to p53 regulated PA26-T2 nuclear protein [H.sapiens]
814	AA609891		EST
815	W86641		EST
816	AA815470		EST
817	AA992324		EST
818	AA446449		EST
819	AI004873		EST
820	AI093982		EST
821	AA393055		ESTs
822	AI168436		ESTs
823	AA809072		ESTs
824	AA926704		ESTs
825	AI183575		ESTs
826	AA121865		ESTs
827	AA725836		ESTs
828	AA621076		ESTs
829	AI018394		ESTs
830	AA885079		ESTs
831	AI148659		ESTs
832	AA460513		ESTs
833	AA758005		ESTs
834	AA868233		ESTs
835	AA488768		ESTs
836	AA496024		ESTs
837	AA496252		ESTs
838	AI339257		ESTs
839	T64080		ESTs
840	AA844729		ESTs
841	AI041148		ESTs
842	AA813319		ESTs
843	AI138555		ESTs
844	AA633536		ESTs
845	AA688025		ESTs
846	U51712		ESTs
847	N50822		ESTs
848	R38569		ESTs
849	AA889533		ESTs
850	AA629398		ESTs
851	AA628190		ESTs
852	AI041289		ESTs
853	AI204513		ESTs
854	AA001410		ESTs
855	AI027500		ESTs
856	AA658107		ESTs
857	AA923244		ESTs
858	AA723819		ESTs
859	AA437069		ESTs

860	AA400934	ESTs
861	M32093	ESTs
862	AA262466	ESTs
863	AA897137	ESTs
864	AA446184	ESTs
865	AA036631	ESTs
866	H86103	ESTs
867	AA401541	ESTs
868	H05826	ESTs
869	AA406039	ESTs
870	AA448082	ESTs
871	AA446064	ESTs
872	H81935	ESTs
873	AA889152	ESTs
874	AI127656	ESTs
875	AI033705	ESTs
876	AI138800	ESTs
877	AI183653	ESTs
878	AA969732	ESTs
879	AI024328	ESTs
880	AA913732	ESTs
881	AA397520	ESTs
882	AI025509	ESTs
883	AA382504	ESTs
884	AI341170	ESTs
885	AA909257	ESTs
886	AA812677	ESTs
887	AA416673	ESTs
888	AA972840	ESTs
889	W31789	ESTs
890	AI261804	ESTs
891	AI091533	ESTs
892	AA991994	ESTs
893	AI024578	ESTs
894	AI040955	ESTs
895	AA953477	ESTs
896	AA846324	ESTs
897	AA417966	ESTs
898	AA150262	ESTs
899	AA724720	ESTs
900	AI031941	ESTs
901	AA620800	ESTs
902	AA813092	ESTs
903	AA101229	ESTs
904	AA025055	ESTs
905	AA382809	ESTs
906	R60655	ESTs, Highly similar to AC005534 2 supported by human ESTs AA412402 [H.sapiens]

907	AA521265	ESTs, Highly similar to AF117065 1 male-specific lethal-3 homolog 1 [H.sapiens]
908	D50640	ESTs, Highly similar to CN3B_HUMAN CGMP-INHIBITED 3',5'-CYCLIC PHOSPHODIESTERASE B [H.sapiens]
909	W44613	ESTs, Highly similar to differentially expressed in Fanconi anemia [H.sapiens]
910	AA400550	ESTs, Moderately similar to ALU4_HUMAN ALU SUBFAMILY SB2 SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]
911	AA648782	ESTs, Moderately similar to GNPI_HUMAN GLUCOSAMINE-6-PHOSPHATE ISOMERASE [H.sapiens]
912	AA496122	ESTs, Moderately similar to KIAA1165 protein [H.sapiens]
913	AI039250	ESTs, Moderately similar to p60 katanin [H.sapiens]
914	AI187883	ESTs, Weakly similar to actin binding protein MAYVEN [H.sapiens]
915	AA865734	ESTs, Weakly similar to AF141326 1 RNA helicase HDB/DICE1 [H.sapiens]
916	D20934	ESTs, Weakly similar to AF148856 1 unknown [H.sapiens]
917	AI434204	ESTs, Weakly similar to Afg1p [S.cerevisiae]
918	AA876372	ESTs, Weakly similar to ALU1_HUMAN ALU SUBFAMILY J SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]
919	AI150114	ESTs, Weakly similar to ALU1_HUMAN ALU SUBFAMILY J SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]
920	AA533191	ESTs, Weakly similar to ALU7_HUMAN ALU SUBFAMILY SQ SEQUENCE

		CONTAMINATION WARNING ENTRY [H.sapiens]
921	AA885514	ESTs, Weakly similar to CAYP_HUMAN CALCYPHOSINE [H.sapiens]
922	AA960902	ESTs, Weakly similar to COXM_HUMAN CYTOCHROME C OXIDASE POLYPEPTIDE VIIB PRECURSO [H.sapiens]
923	AI336338	ESTs, Weakly similar to dJ1108D11.1 [H.sapiens]
924	AI208582	ESTs, Weakly similar to dJ134E15.1 [H.sapiens]
925	AA927467	ESTs, Weakly similar to I38428 T-complex protein 10A [H.sapiens]
926	AA789329	ESTs, Weakly similar to katanin p80 subunit [H.sapiens]
927	AA453640	ESTs, Weakly similar to KCC1_HUMAN CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE TYPE I [H.sapiens]
928	AA744373	ESTs, Weakly similar to KIAA1006 protein [H.sapiens]
929	AA393227	ESTs, Weakly similar to KIAA1016 protein [H.sapiens]
930	AI126471	ESTs, Weakly similar to MRJ [H.sapiens]
931	AA843459	ESTs, Weakly similar to PRP2 MOUSE PROLINE-RICH PROTEIN MP-2 PRECURSOR [M.musculus]
932	R79064	ESTs, Weakly similar to putative type III alcohol dehydrogenase [D.melanogaster]
933	AA708149	ESTs, Weakly similar to Similarity to Human ADP/ATP carrier protein [C.elegans]
934	AA946954	ESTs, Weakly similar to testicular condensing enzyme [M.musculus]
935	AA045194	ESTs, Weakly similar to testicular tektin B1-like protein [H.sapiens]
936	AA223199	ESTs, Weakly similar to Unknown gene product [H.sapiens]

937	AA843452	ESTs, Weakly similar to weak similarity to SP:YAD5 CLOAB [C.elegans]
938	AI224867	ESTs, Weakly similar to zinc finger protein [H.sapiens]
939	AI024879	ESTs, Weakly similar to zona-pellucida-binding protein [H.sapiens]

Table 5 Representative up-regulated genes with known function in testicular seminomas

TS Assignment	Accession No.	Symbol	Gene Name
genes involved in signal transduction pathways			
107	D87116	MAP2K3	mitogen-activated protein kinase kinase 3
97	AA845512	KLF4	Kruppel-like factor 4 (gut)
108	AA583183	MAP4K3	mitogen-activated protein kinase kinase kinase kinase 3
162	AA346311	RAI3	retinoic acid induced 3
163	M29893	RALA	v-ral simian leukemia viral oncogene homolog A (ras related)
120	M13228	MYCN	v-myc avian myelocytomatosis viral related oncogene, neuroblastoma derived
genes involved in oncogenesis			
153	AF045584	POV1	prostate cancer overexpressed gene 1
147	M16750	PIM1	pim oncogene
148	U77735	PIM2	pim-2 oncogene
225	AA465240	VAV2	vav 2 oncogene
170	X12949	RET	ret proto-oncogene
genes involved in cell cycle			
20	AA682870	CCND2	cyclin D2
25	M81934	CDC25B	cell division cycle 25B
genes involved in cell adhesion and cytoskeleton			
92	Z68228	JUP	junction plakoglobin
45	AA128470	DSP	desmoplakin (DPI, DPII)
26	X63629	CDH3	cadherin 3, type 1, P-cadherin (placental)
96	U06698	KIF5A	kinesin family member 5A

Semi-quantitative RT-PCR

Twenty nine up-regulated genes were selected and their expression levels examined by applying the semi-quantitative RT-PCR experiments. A 3-μg aliquot of aRNA from each

sample was reverse-transcribed for single-stranded cDNAs using random primer (Roche) and Superscript II (Life Technologies, Inc.). Each cDNA mixture was diluted for subsequent PCR amplification with the same primer sets that were prepared for the target DNA- or α-tublin-specific reactions. The primer sequences are listed in Table 2. Expression of α-tublin served as an internal control. PCR reactions were optimized for the number of cycles to ensure product intensity within the linear phase of amplification. Comparing the ratios of the expression levels of the 29 up-regulated genes (CCND2, GIP, H1F2, NMA, PIM2, POV1, PRDM4, PTMS, RAI3, PYPAF3, T1A-2, TCOF1, TGIF2, FLJ10713, FLJ20069, KIAA0456, KIAA1198, DKFZp434K0621, EST(270), FLJ13352, FLJ12195, EST(285), NCOA6IP, EST(295), PLXNA2, EST(311), EST(320), LOC152217, EST(341)) whose expression were overexpressed in almost of all informative cases, the results were highly similar to those of the microarray analysis in the great majority of the tested cases (Fig. 1,Fig.2A).

Table 2: Primer Sequence for RT-PCR

TS Assignment	GENE	Forward Primer	SEQ ID NO	Reverse Primer	SEQ ID NO
20	CCND2	5'-TGATCAGTGTATGCGAAAAGGT-3'	1	5'-GGTCAAGGTGAGTTTATTGTCCA-3'	2
59	GIP	5'-TTGCCATGGACAAGATTCAC-3'	3	5'-TTGTCTGATCCAGCAAGCAG-3'	4
70	H1F2	5'-CGGAACCAAACCTAAGAAGC-3'	5	5'-CTTCACAGCCTTAGCAGCACTT-3'	6
130	NMA	5'-CCTCTGCAAACAGAATCTTG-3'	7	5'-AAGATGTAGAAGCTTACATAGGGCA-3'	8
148	PIM2	5'-GGAAATAAGGCTTGCTGTTTGT-3'	9	5'-AATAGTGGGTTTCCACACATGG-3'	10
153	POV1	5'-CACAACATGCAATGTGTCTGTG-3'	11	5'-TCCTCTAAGACTTGCAAGCAGC-3'	12
156	PRDM4	5'-CATGAAGGAAAACGGGATTATG-3'	13	5'-GTGCAGAAAGAGACTCATCCG-3'	14
159	PTMS	5'-TCCCACCTAACCTCTGCATC-3'	15	5'-GAAGCGCGACCATTTCTTTA-3'	16
162	RAI3	5'-GGCTGATACTTCTCTCATCTTGC-3'	17	5'-GCCACCACATCTTTATTGCATAC-3'	18
171	PYPAF3	5'-TGGGGTTCTAAGACAAAGAACTG-3'	19	5'-GTGAGAAAACCAGTGTCAAATCC-3'	20
209	T1A-2	5'-TGCTGGTGCTATTTACTGACGTA-3'	21	5'-AAAAGACCGTTTCTGACTCTGTG-3'	22
212	TCOF1	5'-AAGTGACCTCCTCTCCTTCC-3'	23	5'-CACCCTTCCTCCAAGTCTTTTAT-3'	24
214	TGIF2	5'-GAACCCAGTGGATGTAACAGAAC-3'	25	5'-TACTGCAGAGACTTAGCTGGTCC-3'	26

240	FLJ10713	5'-ACTTATAGTCCTGCGAGTCTGGG-3'	27	5'-GGCAGGAGAGAAGAACATCTTG-3'	28
244	FLJ20069	5'-CATCTCCTTTGTTTCGATAGGA-3'	29	5'-GATCACTGTGGGTCTTAAGCAA-3'	30
253	KIAA0456	5'-GGGCTGGTGCAGATCTACTT-3'	31	5'-TCCAACATCTGTTGAGTGACAGT-3'	32
259	KIAA1198	5'-CACTCAGAATTCTTACCTCCCCT-3'	33	5'-GTGATGTGAAGCAAGGTAGTTCC-3'	34
267	DKFZp434K0621	5'-GCCAAAAATGGCTCTCTAGG-3'	35	5'-CAGACACGCACTTGTGGTTTATT-3'	36
270	EST	5'-GTGTCCACTTAGAGCCTCACG-3'	37	5'-ATCCTTCTTCCTATACTTCCCCC-3'	38
278	FLJ13352	5'-TTTAATCAGGCCCTGTCTGC-3'	39	5'-GGGGTATAGAAATGGAATGGAGA-3'	40
282	FLJ12195	5'-CTGGAAGAAGAAGGAACAGGTCT-3'	41	5'-GGTTGCTGAGATTTTATCTGTGG-3'	42
285	EST	5'-CAAATGCTCTGCTTTGTACTCCT-3'	43	5'-CATGAATGAGCCTGAAATAGTCC-3'	44
287	NCOA6IP	5'-CGGGAGGATTGTAAGATACTGTG-3'	45	5'-ACTTCTCATGAGTTCAGCCTCAG-3'	46
295	EST	5'-GTAGATGTGGGGACAACAGAGAG-3'	47	5'-TTTAAAGTCACCTTAGGTTGGGG-3'	48
303	PLXNA2	5'-GTTTTTGTGGGGACTAAGAGTG-3'	49	5'-GGAGGAAGTAGCTAGAAGCTAAG-3'	50
311	EST	5'-CTTTTCCCACAAGAACCATTTC-3'	51	5'-CTGGTGTAATCAGACACCACGTA-3'	52
320	EST	5'-CTCATCTGTACCCTCACTGGGAT-3'	53	5'-CTAAAGTCTCCCAGTTTCCCCT-3'	54
337	LOC152217	5'-AAGCCAGAGAGCCTTTCCTC-3'	55	5'-CGGTATTCTTAACACATCTTGCC-3'	56
341	EST	5'-ACCTAACGTTTGTGCCTTATGTG-3'	57	5'-AGGTTGGAAGATCCATTTCCTT-3'	58
	TUBA	5'-CTTGGGTCTGTAACAAAGCATTC-3'	59	5'-AAGGATTATGAGGAGGTTGGTGT-3'	60
	β2MG	5'-TTAGCTGTGCTCGCGCTACT-3'	61	5'-TCACATGGTTCACACGGCAC-3'	62

EXAMPLE 3: GROWTH-INHIBITORY EFFECTS OF SIRNA DESIGNED TO REDUCE EXPRESSION OF PYPAF3

Through analysis of genome-wide expression profiles by a cDNA microarray, we have applied to isolate novel molecular targets for diagnotic tumor markers, treatments and prevention of testicular germ cell tumor. Among the genes that commonly up-regulated in testicular seminomas, we focused on PYRIN-containing Apaf-1-like protein 3 (PYPAF3(NM_139176)) that were significantly up-regulated in 7 of 8 cases with testicular seminomas, compared to normal human organ including testis, heart, lung, liver, kidney, brain and bone marrow by semi-

quantitative RT-PCR analysis. Although we identified PYPAF3 as up-regulated gene in testicular seminona at present (bulid #160), we initially listed this gene up as RMP:RMB5-mediating protein through expression profiles using cDNA microarray representing 23,040 genes that were retrieved from Unigene database (build #131) on National Center for Biotechnology Information.

Multiple-tissue Northern blot analysis using *PYPAF3* cDNA fragment as a probe revealed a transcript of approximately 3.3kb that was expressed only in testis. Immunocytocheminal study revealed PYPAF3 protein was present throughout the cytoplasm. Transfection of small interference RNA (siRNA) of *PYPAF3* inhibited the expression of mRNA of *PYPAF3* and cell growth of testicular germ cell tumor cells. These findings suggest that *PYPAF3* might be involved in tumorigenesis of testicular seminomas , and represents a promising candidate for development of targeted therapy for testicular germ cell tumors.

Cell lines and tissue specimens

COS-7 cells and Tera-2 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD). All cell lines were grown in monolayers in appropriate media supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic solution (Sigma, St. Louis, MO), Dulbecco's modified Eagle's medium (Sigma) for COS-7 McCoy's 5A (Invitrogen, Carlsbad CA), and maintained at 37°C in humid air containing 5% CO_2.

Semi-quantitative RT-PCR

Normal human testis, heart, lung, kidney, liver, brain, and bone marrow poly(A)$^+$ RNA were obtained by Clontech (Palo Alto, CA). A 3-μg aliquot of amplified RNA from each sample was reverse-transcribed to single-stranded cDNAs using random primer (Roche) and Superscript II reverse transcriptase (Invitrogen). Each single-strand cDNA was diluted for subsequent PCR amplification. Standard RT-PCR procedures were carried out in 20ml volumes of PCR buffer (Takara, Kyoto, Japan), and amplified for 5min at 94°C for denatureing, followed by 22 (for TUBA3) or 31 (for PYPAF3) cycles of 94°C for 30sec, 55°C for 30sec and 72°C for 30sec. Primer sequences were as follows: for TUBA3, forward 5'-CTTGGGTCTGTAACAAAGCATTC-3'(SEQ ID NO:59), and reverse 5'-AAGGATTATGAGGAGGTTGGTGT-3'(SEQ ID NO:60); for PYPAF3, forward 5'-TGGGGTTCTAAGACAAAGAACTG-3' (SEQ ID NO:19), and reverse 5'-GTGAGAAAACCAGTGTCAAATCC-3' (SEQ ID NO:20).

Northern blot analysis

Human multiple-tissue blots (Clontech) were hybridized with a ^{32}P-labeled *PYPAF3* cDNA fragment as a probe. The cDNA was prepared by RT-PCR as described above. Pre-hybridization, hybridization and washing were performed according to the supplier's recommendations. The blots were autoradiographed with intensifying screens at –80°C for 7 days.

Immunocytocheminal staining

The entire coding region of *PYPAF3* was amplified by RT-PCR using forward primer 5'-CGCGGATCCCACTATGACATCGCCCCAGC-3' (SEQ ID NO:63) and reverse primer 5'-CCGCTCGAGGCAAAAAAAGTCACAGCACGG-3' (SEQ ID NO:64). After the PCR product was digested with BamH1 and Xho1, it was cloned into an appropriate cloning site of plasmid vector pcDNA3.1-myc/His (Invitrogen). COS7 cells were transfected with pcDNA3.1(+)-PYPAF3-myc/His mixed with FuGene6 transfection reagent (Roche, Basel, Switzerland). COS7-derived transiently transfectants were washed twice with PBS(-), fixed with 4% paraformaldehyde solution for 15 min at 4°C, and rendered permeable with PBS(-) containing 0.1% Triton X-100 for 2.5 min. Cells were covered with 3% BSA in PBS(-) for 60 min to block non-specific antibody-binding sites prior to reaction with the primary antibody. PYPAF3 protein was detected with mouse anti-human c-Myc 9E10 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) as primary and goat anti-mouse FITC (Jackson ImmunoResearch, West Grove, PA) as secondary antibody. Nuclei were counterstained by 4',6'-diamidine-2'-phenylindole dihydrochloride (Vector Laboratories, Burlingame, CA). Fluorescent images were obtained with an Eclipse E800 microscope (Nikon, Tokyo, Japan).

Treatment of testicular germ cell tumor cells with Small interference RNA (siRNA)

Transcription of the U6RNA gene by RNA polymerase III produces short transcripts with uridines at the 3' ends. We amplified a genomic fragment containing the promoter region of U6RNA by PCR, using primers 5'-TGGTAGCCAAGTGCAGGTTATA-3'(SEQ ID NO:65), and 5'-CCAAAGGGTTTCTGCAGTTTCA-3'(SEQ ID NO:66) and human placental DNA as a template. The product was purified and cloned into pCR2.1 plasmid vector using a TA cloning kit, according to the supplier's protocol (Invitrogen). The BamHI, XhoI fragment containing U6RNA was purified and cloned into pcDNA3.1(+) between nucleotides 56 and 1257, and the fragment was amplified by PCR using primers 5'-

TGCGGATCCAGAGCAGATTGTACTGAGAGT-3'(SEQ ID NO:67) and 5'-CTCTATCTCGAGTGAGGCGGAAAGAACCA-3'(SEQ ID NO:68). The ligated DNA became the template for PCR amplification with primers 5'-TTTAAGCTTGAAGACCATTTTTGGAAAAAAAAAAAAAAAAAAAAAACA-3'(SEQ ID NO:69) and 5'-TTTAAGCTTGAAGACATGGGAAAGAGTGGTCTCA-3'(SEQ ID NO:70). The product was digested with HindIII and subsequently self-ligated to produce a psiU6BX vector plasmid. SiRNA expression vectors against PYPAF3 (psiU6BX-PYPAF3) and control plasmids (psiU6BX-EGFP, psiU6BX-Luciferace) were prepared by cloning double-stranded oligonucleotides following as Table 6 into the BbsI site in the psiU6BX vector. Each siRNA expression vector was transfected with Fugene6 (Roche) into testicular germ cell tumor line Tera-2 which expressed PYPAF3 endogenously. After selection by Geneticin (Invitrogen), cell proliferation was evaluated after two weeks by colony formation assay using Giemsa staining and after one week by Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) (39). A knockdown effect of PYPAF3 mRNA was identified by semi-quantitative RT-PCR.

Confirmation of expression of PYPAF3in testicular seminomas by semi- quantitative RT-PCR.

We have been using a cDNA microarray to analyze gene-expression profiles of 23,040 genes in testicular seminomas from 13 patients (12). Among the up-regulated genes, we focused on PYPAF3, which was overexpressed in 7 of 8 informative cases whose signal intensities of the gene were higher than the cut-off in patients with testicular seminomas. Furthermore, we performed semi-quantitative RT-PCR analysis and then confirmed elevated expression of PYPAF3 in 7 of 8 testicular seminomas, compared to normal human testis, heart, lung, liver, kidney, brain and bone marrow (Figure 2A).

Multiple-tisssue Northern blot analysis and sub-cellular localization of PYPAF3 protein

Northern analysis using *PYPAF3* cDNA fragment as a probe (see Material and Method) revealed a transcript of approximately 3.3kb that was expressed only in testis (Figure 2B). Furthermore, to investigate the role of PYPAF3 protein in mammalian cells, we constructed a plasmid to express myc-tagged PYPAF3 protein (see Material and Method). When the plasmid DNA was transiently transfected into COS-7 cells, the tagged PYPAF3 protein was present throughout the cytoplasm of transfected cells (Figure 3).

Growth-inhibitory effects of small-interference RNA (siRNA) designed to reduce expression of

PYPAF3

To assess the growth-promoting role of PYPAF3, we knocked down the expression of endogenous PYPAF3 in testicular germ cell tumor line Tera-2 cells, by means of the mammalian vector-based RNA interference (RNAi) technique and examined the effect on cell growth (see Materials and Methods). As shown in Figure 4a, introduction of psiU6BX-PYPAF3 *(Si 4)* clearly reduced expression of PYPAF3 transcript in Tera-2 cell lines while no effect was observed in cells transfected with control plasmids (psiU6BX-*EGFP* and psiU6BX-Luciferase siRNA expression vectors). To confirm the gene-specific growth reduction by psiU6BX-PYPAF3, we performed colony-formation assays of the same two cell lines; as shown in Figure 4b and 4c, introduction of psiU6BX- PYPAF3 *(Si 4)* significantly suppressed growth of Tera-2 cells, consisting with the result of above reduced expression, whereas introduction of *Si 3* markedly suppressed growth of Tera-2 cells, although knock down of PYPAF3 transcript level showed no almost of reduction. Moreover, MTT assays also indicated significantly growth inhibition of Tera-2 cells when PYPAF3 expression was repressed using psiU6BX-PYPAF3 *(Si 3 and Si 4)* (Figure 4a, b). Each result was verified by three independent experiments.

Table6. Oligonucleotides sequences for small interference RNA of *PYPAF3*

			SEQ ID NO
Si1	Sense	5'-CACCGAGGCTGATGGCAAGAAACT TCAAGAGAGTTTCTTGCCATCAGCCTC-3'	71
	Antisense	5'-AAAAGAGGCTGATGGCAAGAAACT CTCTTGAAGTTTCTTGCCATCAGCCTC-3'	72
Si2	Sense	5'-CACCGAGATGAATCTCACGGAATTT CAAGAGAATTCCGTGAGATTCATCTC-3'	73
	Antisense	5'-AAAAGAGATGAATCTCACGGAATTC TCTTGAAATTCCGTGAGATTCATCTC-3'	74
Si3	Sense	5'-CACCGTAGGACACTTCTTATTCGTT CAAGAGACGAATAAGAAGTGTCCTAC-3'	75
	Antisense	5'-AAAAGTAGGACACTTCTTATTCGT CTCTTGAACGAATAAGAAGTGTCCTAC-3'	76
Si4	Sense	5'-CACCGTGATGCATTGTTCCTTCATT CAAGAGATGAAGGAACAATGCATCAC-3'	77
	Antisense	5'-AAAAGTGATGCATTGTTCCTTCATC TCTTGAATGAAGGAACAATGCATCAC-3'	78
Si5	Sense	5'-CACCGCTTGGCTGTAGATATCTCTT CAAGAGAGAGATATCTACAGCCAAGC-3'	79
	Antisense	5'-AAAAGCTTGGCTGTAGATATCTCTC TCTTGAAGAGATATCTACAGCCAAGC-3'	80
SiEGFP	Sense	5'-CACCGAAGCAGCACGACTTCTTCT	81

		TCAAGAGAGAAGAAGTCGTGCTGCTTC-3'	
		5'-AAAAGAAGCAGCACGACTTCTTCTCT	
	Antisense	CTTGAAGAAGAAGTCGTGCTGCTTC-3'	82
SiLuciferac		5'-CACCGTGCGCTGCTGGTGCCAACT	
e	Sense	CTCTTGAAGTTGGCACCAGCAGCGCAC-3'	83
		5'-AAAAGTGCGCTGCTGGTGCCAACTT	
	Antisense	CAAGAGAGTTGGCACCAGCAGCGCAC-3'	84

Industrial Applicability

The gene-expression analysis of TS described herein, obtained through a combination of laser-capture dissection and genome-wide cDNA microarray, has identified specific genes as targets for cancer prevention and therapy. Based on the expression of a subset of these differentially expressed genes, the present invention provides a molecular diagnostic markers for identifying or detecting TS.

The methods described herein are also useful in the identification of additional molecular targets for prevention, diagnosis and treatment of TS. The data reported herein add to a comprehensive understanding of TS, facilitate development of novel diagnostic strategies, and provide clues for identification of molecular targets for therapeutic drugs and preventative agents. Such information contributes to a more profound understanding of testicular tumorigenesis, and provide indicators for developing novel strategies for diagnosis, treatment, and ultimately prevention of TS.

All patents, patent applications, and publications cited herein are incorporated by reference in their entirety. Furthermore, while the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope of the invention.

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